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VOCAL FUNCTION FOLLOWING VERTICAL HEMILARYNGECTOMY: COMPARISON OF FOUR RECONSTRUCTION TECHNIQUES IN THE CANINE

ROBERT J. ANDREWS, MD JOEL A. SERCARZ, MD MING YE, MD
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LOS ANGELES, CALIFORNIA

The goals of laryngeal reconstruction have been prevention of aspiration, production of a functional voice, and maintenance of an adequate airway for decannulation. A number of procedures for partial laryngeal reconstruction have accomplished these objectives. However, few studies have attempted to compare patients' vocal characteristics following different reconstruction procedures. In this study, an in vivo canine model was used to compare acoustic and aerodynamic measures of vocal function for the following vertical hemilaryngectomy reconstruction techniques: 1) a superiorly based sternohyoid muscle flap, 2) a modified epiglottic laryngoplasty, 3) a new procedure using a layered vascularized buccal mucosal flap and a transversely oriented sternohyoid muscle flap, and 4) hemilaryngeal transplantation combined with arytenoid adduction. Hemitransplantation provided the most efficient phonation of the four techniques. The vascularized buccal mucosa flap produced the best phonation of the autologous tissue techniques examined. Both vascularized buccal mucosa flap and hemilaryngeal transplantation subjects demonstrated a mucosal wave on stroboscopy. The results indicate that vocal function will improve as the layered structure of the vocal fold is more accurately replicated in a reconstructed hemilarynx. Endoscopic findings and whole organ sections are presented.

KEY WORDS — laryngeal reconstruction, vertical hemilaryngectomy, vocal function.

INTRODUCTION

Although surgeons have described partial laryngeal reconstruction techniques for over a century, few studies have attempted to compare laryngeal function following reconstruction with different methods. In this study, four different methods for reconstruction of vertical hemilaryngectomy defects were evaluated in an in vivo canine model: 1) a sternohyoid muscle flap, 2) an epiglottic cartilage and mucosa composite flap with a separate sternohyoid muscle flap, 3) a vascularized buccal mucosal flap and underlying sternohyoid muscle flap with laryngeal framework reconstruction, and 4) a hemilaryngeal transplant with arytenoid adduction. We hypothesized that closer replication of the structural properties of the vocal fold would result in corresponding improvements in vocal function.

Reconstruction after partial laryngectomy is intended to prevent aspiration, provide a functional voice, and maintain an airway adequate for decannulation. Thus, an ideal reconstruction technique should restore the sphincteric competence of the glottis; if the arytenoid is resected, it should provide a relatively rigid posterior structure to occlude against the intact arytenoid, producing glottal competence. Closure of the glottis should be firm, so that an adequate subglottic pressure can be produced to drive phonation. Finally, reconstruction should provide a cross-sectional lumen sufficient for respiration. An adequate airway is more likely when one arytenoid can be preserved.

Two types of mucosal flaps have been used in laryngeal reconstruction. Rotational flaps are generally taken from the false vocal fold, aryepiglottic fold, and pyriform sinus. Free mucosal grafts are usually harvested from the oral mucosa. These flaps are intended to replace the excised laryngeal mucosa and lamina propria, which function as a superior glottic interface in the native state. However, such flaps have several disadvantages. First, if used alone, they do not provide enough bulk to replace the excised tissue, and therefore are usually combined with underlying muscle or cartilaginous flaps. Second, rotational flaps often produce distortion of the pyriform sinus anatomy, potentially resulting in aspiration. Third, free mucosal grafts placed in the larynx typically undergo atrophy or necrosis. This atrophy may result in an incompetent glottis. Finally, such

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From the Division of Head and Neck Surgery, University of California, Los Angeles School of Medicine, Los Angeles, California. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Los Angeles.

Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 4-5, 1996.

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grafts ultimately do not differ histologically from muscle and myofascial surfaces that gradually mucosalize.[1,2] Therefore, many investigators believe the disadvantages of laryngeal reconstruction with rotational or free mucosal grafts outweigh their benefits.

Pedicled muscle flaps are much more commonly used for reconstruction of partial laryngectomy defects.[1,3-6] Potential donor muscles include the sternohyoid, omohyoid, and sternocleidomastoid. Some methods also include the overlying fascia or thyroid perichondrium. The muscle can be pedicled superiorly or inferiorly, or placed as a bipedicled flap.

Muscle flaps provide excellent bulk to replace the excised hemilarynx and can be modified for various tissue defects. However, they also result in an adynamic glottic interface with a thin mucosal layer that has poor viscoelastic and vibratory properties. Despite the disadvantages related to vocal function, many otolaryngologists contend that muscle flaps are the best option available for partial laryngeal reconstruction because of their versatility and consistent results.[4]

A wide variety of cartilaginous flaps have been described for partial laryngeal reconstruction.[7-10] One approach utilizes a composite epiglottic flap with attached mucosa for reconstruction of near-total or vertical hemilaryngectomy defects.[11-14] In this method, the mucosa provides an intact laryngeal surface, while the epiglottic cartilage contributes structural support.

A few free tissue transfer methods for laryngeal reconstruction have also been described. Cases of human laryngeal reconstruction with iliofemoral, deltopectoral,[15] or radial forearm free flaps[16] have been reported, and reconstruction using a lateral thoracic fascia vascularized flap with free buccal mucosa and cartilage grafts has been described in the rabbit model.[17] To date, free tissue transfers have not been widely used for partial laryngeal reconstruction.

The present hypothesis is that vocal function will improve as the layered structure of the vocal fold is more accurately replicated in a reconstructed hemilarynx. A canine model was used to test the hypothesis. Modified muscle and cartilage flaps were chosen as the first two reconstruction methods for evaluation. The third technique was a pedicled mucosal flap with intact, vascularized submucosa. We hypothesized that this technique would provide more efficient phonation than either the muscle or cartilage flap, because it more closely replicates the native laryngeal lamina propria. A vascularized buccal mucosal flap was chosen because of its reliable blood supply. For replacement of the laryngeal musculature, a transversely oriented, superiorly based sternohyoid muscle flap was placed at the level of the glottis. Finally, a microplate was fixed parallel to the neoglottis to stabilize the laryngeal framework where the thyroid cartilage had been removed.

The fourth technique, hemilaryngeal transplantation, is the most anatomically accurate method of hemilaryngeal reconstruction, and thus represents a theoretic ideal. In addition, it provides an opportunity to investigate the feasibility of partial organ transplantation. Reinnervation of the thyroarytenoid muscle and an arytenoid adduction were included in this method.

MATERIALS AND METHODS

OVERVIEW OF EXPERIMENTAL DESIGN

Twelve adult male mongrel dogs were selected for the experiment. Three animals were assigned at random to each of three autologous reconstruction conditions; the remaining three subjects were retained as replacements for animals developing serious postoperative complications. Each animal was approximately 1 year old and weighed 20 to 25 kg. All had normal-appearing vocal folds on direct laryngoscopy; dogs with an unusually large posterior glottic gap on laryngeal closure were excluded.

Two additional animals (male littermate beagles, approximately 1 year old and weighing 12 to 15 kg) were assigned to the hemilaryngeal transplantation condition. Mixed leukocyte cultures and genetic sequencing were performed to assess the major histocompatibility reactivity at the DLA-D region and verify a haplo-identical match.

Prior to laryngeal reconstruction, phonation was produced with transtracheal stimulation to accumulate baseline acoustic, aerodynamic, and videolaryngoscopic data. A stimulating electrode endotracheal tube was inserted through a tracheotomy. Following the procedure, the tracheotomy was closed and allowed to heal for 4 to 6 weeks prior to hemilaryngectomy and reconstruction.

When recovery was complete, a surgical intervention (hemilaryngectomy with autologous reconstruction, or hemilaryngeal transplantation) was performed. Two months following surgery, phonation was again evaluated with transtracheal stimulation. Laryngeal specimens were harvested after the second evaluation.

BASELINE ACOUSTIC, AERODYNAMIC, AND ENDOSCOPIC DATA

Preparation for Phonation. Each dog received acepromazine maleate (0.2 mg/kg) intramuscularly

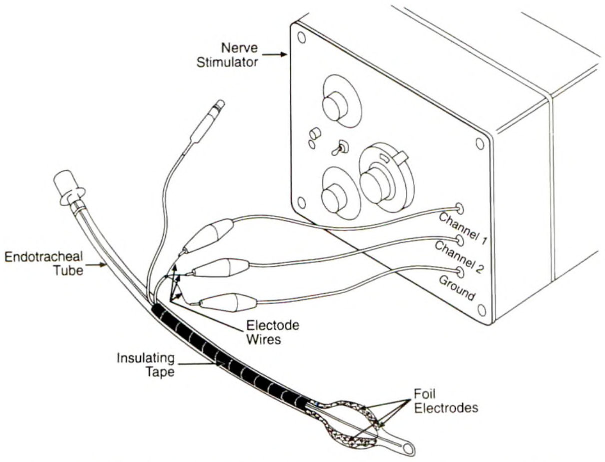

Fig 1. Custom endotracheal tube and nerve stimulator for transtracheal recurrent laryngeal nerve (RLN) stimulation.

as a preoperative sedative. Pentobarbital sodium was administered intravenously to the level of corneal anesthesia. Additional pentobarbital was given to maintain this level of anesthesia throughout the procedure.

The subject was placed supine on the operating table and direct laryngoscopy was performed. The dog was intubated with a cuffed endotracheal tube for ventilator-assisted respiration. Cefazolin sodium (1.0 g) was administered intravenously for antimicrobial prophylaxis and continued for 7 days postoperatively. Dexamethasone sodium phosphate (8 mg intravenously) was administered to reduce laryngeal edema during phonation.

Following sterile skin preparation, a midline vertical incision was made from the sternal notch to just below the cricoid cartilage. Dissection was carried out in the midline and the strap muscles were retracted laterally. A low tracheotomy was made at the level of the sternal notch, through which a cuffed endotracheal tube was passed for assisted ventilation. The oral endotracheal tube was then removed.

A second tracheotomy was placed three tracheal rings superior to the first, and a recurrent laryngeal nerve (RLN)–stimulating endotracheal tube was inserted and directed superiorly so that its tip was located approximately 10 cm below the glottis. This tube (Fig 1) had foil electrodes attached to the cuff so that the RLN could be stimulated through the tracheal cartilage.[18,19] The tube was positioned so that the electrodes on the cuff were oriented with the stimulating electrodes adjacent to the tracheal-esophageal groove. A ground electrode was positioned at the anterior tracheal wall.

Recurrent Laryngeal Nerve Stimulation. With transtracheal stimulation of the RLN, phonation could be produced in each dog without risk of injury to the RLN. (Superior laryngeal nerve stimulation would have required an open dissection and was not performed.) Stimulation was provided via a nerve stimulator (WR Medical Electronics Co, model 2SLH, St

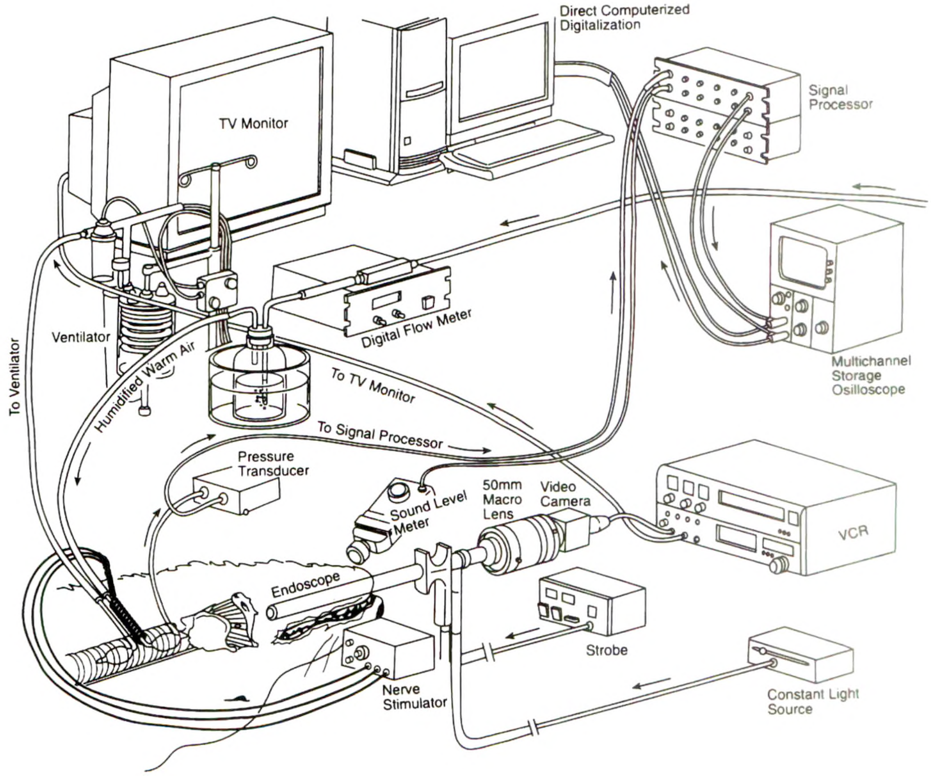

Fig 2. Schematic diagram of equipment used for collection of transtracheal phonation data.

Paul, Minn) connected to the tracheal electrodes. The stimulus frequency was 80 Hz, with a 1.5-millisecond pulse duration and current of 3 to 5 mA. The stimulator and other equipment used in the in vivo model of phonation are depicted in Fig 2.

Subglottic Pressure, Flow, and Acoustic Signal. Subglottic pressure was measured with a catheter-tipped pressure transducer (Millar Instruments, model SPC 340, Houston, Tex) placed 2.0 cm below the glottis. The instrument was calibrated with a manometer prior to each experiment. The pressure-regulating system exit port was connected to a flowmeter (Edwards High Vacuum International, model 825 Series B, Wilmington, Mass). Air was bubbled through 5 cm of heated water for humidification and warming to 37°C when measured at the glottic outlet. This flow was directed through the glottis to drive phonation during transtracheal stimulation of the RLN.

Sound level measurements were made in decibels sound pressure level with an acoustic meter (Quest Electronics, model 211A, Oconomowoc, Wis) that was suspended at the entrance to the oral cavity.

Subglottic pressure, flow, and acoustic signals were digitized simultaneously with a 12-bit analog-to-digital converter (SA Instrumentation, model SA-414, San Diego, Calif) and C-Speech signal processing software (Paul Milenkovic, University of Wisconsin, Madison, Wis). The signals were low-pass-filtered at 3 kHz and sampled at 20 kHz for 2.8 seconds in each trial.

The acoustic power and aerodynamic power were calculated with the following formulae.

(1) Acoustic power = $2r^2Pe^2/Po^c$

where: r = sound meter distance to the glottis in cm

Pe = acoustic intensity in dynes/cm^2

$dB = (20\log_{10})Pe/0.0002$ dynes/cm^2

$Po^c = 41.4$ dynes/cm^3

(2) Aerodynamic power = $Fm\ Sm$

where: Fm = flow in cm^3/s

Sm = subglottic pressure in dynes/cm^2

1 mm Hg = 1.3332×10^{-3} dynes/cm^2

Videolaryngoscopy and Videostroboscopy. All subjects underwent an endoscopic evaluation during phonation. Videolaryngoscopy was performed with a 0° endoscope (Karl Storz Endoscopy-America Inc, Culver City, Calif) connected to a halogen light source via a fluid-filled light cable. For videostroboscopy, a laryngostrobe (Storz, model 8000) was connected to the endoscope. Images were recorded with a charge-coupled device video camera (Toshiba, model IK-C30A, Buffalo Grove, Ill) and a $^3/_4$-in videotape recorder (Sony, model VO-9850, Park Ridge, NJ). Recorded images were viewed on a video monitor (Sony, model PVM-1341) for frame-by-frame analysis.

Test-Retest Reliability. To ensure that preoperative measures of phonation were truly representative, baseline measures were replicated for one subject prior to hemilaryngectomy. In this subject, phonation was produced with transtracheal stimulation, as described above; after an interval of 1 month for recovery, the in vivo model was reapplied and a second set of baseline data were gathered. No significant differences were found between the two pretests for any measure of phonation.

HEMILARYNGEAL RESECTION TECHNIQUE

The subjects received acepromazine maleate (0.2 mg/kg) intramuscularly as a preoperative sedative. Pentobarbital sodium was administered intravenously to the level of corneal anesthesia. The subject was placed supine on the operating table and a mixture of 0.5% to 2.0% halothane and oxygen was administered to maintain anesthesia throughout the procedure. Ampicillin and gentamicin were administered intravenously as antimicrobial prophylaxis and continued for 7 days postoperatively. Intraoperative monitoring included a continuous electrocardiogram and a rectal thermometer.

Following sterile skin preparation, a tracheotomy was performed at the fifth tracheal ring with an inferiorly based flap sutured to the skin edge. The oral endotracheal tube was removed and a sterile cuffed endotracheal tube was placed in the stoma for assisted ventilation. A midline vertical skin incision was made that extended from the level of the hyoid bone to the cricoid cartilage.

A pedicled external thyroid perichondrium flap was made. The perichondrium was incised vertically a few millimeters lateral to the midline on the contralateral side of the specimen and horizontally along the superior and inferior borders of the thyroid cartilage from the vertical incision to 5 mm anterior to the posterior edge of the ipsilateral thyroid cartilage. An elevator was used to raise the perichondrial flap off the underlying cartilage. A No. 15 blade was used to make the thyroid cartilage cuts in the midline and at the border of the posterior third and anterior two thirds on the ipsilateral side of resection. A midline thyrotomy was made and superior and inferior cuts were completed through the thyrohyoid and cricothyroid membranes. A mucosal incision was made in the midline of the posterior commissure. The specimen was excised, including the true vocal fold, ventricle, false vocal fold, thyroarytenoid muscle, arytenoid, and anterior two thirds of the ipsilateral thyroid cartilage.

RECONSTRUCTION TECHNIQUES

Superiorly Based Muscle Flap. This procedure has been described in detail by Calcaterra.[4] Briefly, the sternohyoid muscle flap, including the outer layer of fascia, was made larger than the dimensions of the laryngeal defect in anticipation of muscle atrophy. The flap was raised superiorly to the hyoid bone with preservation of the vascular and nerve supply. The flap was rotated with the external fascial layer directed toward the laryngeal lumen. The fascia was sutured to the laryngeal mucosa posteriorly and inferiorly along the cricoid with interrupted absorbable sutures. The perichondrium was then approximated as described, with the anterior remnant of the muscle flap used to reconstruct the anterior commissure.

Epiglottic Cartilage and Mucosa Composite Flap. The epiglottis was mobilized as described by Tucker et al.[11] The preepiglottic contents were dissected in a plane immediately anterior to the cartilage. The hyoepiglottic and glossoepiglottic ligaments were cut. The superior mucosal attachments of the epiglottis were carefully preserved to provide a pedicled vascular supply to the cartilage and mucosa on its laryngeal surface. The composite flap was then brought into the larynx and the inferior and lateral edges were trimmed to fit in the defect. The flap was sutured to the cricoid and free mucosal edge posteriorly and to the cricothyroid membrane inferiorly as described by Olson and Sullivan.[14]

A superiorly based sternohyoid muscle flap was mobilized, placed lateral to the epiglottic composite flap, and sutured to the cricoid inferiorly. The muscle flap provided bulk for medialization of the epiglottic flap. For anterior closure, the mucosa and inner perichondrium of the epiglottic flap and thyroid cartilage were approximated with interrupted 5-0 absorbable sutures. The external thyroid perichondrium was then approximated over both flaps.

Vascularized Buccal Mucosa Flap. A third group of dogs was reconstructed with a vascularized buccal mucosa flap. The details of this technique will be published separately. Briefly, a 2 × 3-cm pedicled flap was elevated, based on the facial artery and vein. This was sutured into position in the hemilarynx. A transversely oriented, superiorly based sternohyoid muscle flap, as described by Hirano,[1] was positioned deep to the mucosal flap to provide adequate bulk for the neoglottis. A microplate fixed to the thyroid cartilage served both to stabilize the laryngeal framework and as lateral support for the mucosal and muscle flaps at the level of the glottis.

Hemilaryngeal Transplantation With Arytenoid Adduction. Many aspects of the hemilaryngeal transplant protocol, including canine selection, immunosuppression, surgical preparation, and postoperative care, were identical to those used for orthotopic canine laryngeal transplantation.[20] Briefly, the vascular supply of the hemitransplant was based on the cranial thyroid artery and hyoid venous arch. The anterior branch of the recipient RLN was dissected and tagged for anastomosis to the thyroarytenoid branch in the donor prior to removal of the specimen. The intralaryngeal defect and donor specimen were otherwise identical to those of the subjects who underwent autologous reconstructions. The donor arytenoid was fixed to the recipient cricoid with nonabsorbable suture and an arytenoid adduction was performed. The mucosa was closed in layers and miniplates were used to stabilize the laryngeal framework at the anterior commissure and posterior third of the thyroid cartilage.

POSTOPERATIVE CARE

Autologous Reconstructions. Each dog was given water on the first postoperative day, and soft food on the third postoperative day. All of the subjects were decannulated between the second and third postoperative weeks. The dogs were decannulated if they tolerated a sustained period of exercise with the tracheotomy tubes removed and their stomas occluded with surgical tape. After decannulation, the stomas closed spontaneously.

Hemilaryngeal Transplant. The postoperative care was as described by Berke et al,[20] except that an esophagostomy tube was utilized to provide nutrition as soon as bowel function returned. This was removed on postoperative day 7 and the animal was given water and soft food the following morning. The dog was not decannulated, so that the transplant hemilarynx could be evaluated with a flexible endoscope placed through the tracheotomy stoma. In this manner, the hemilarynx was monitored without sedation for gross signs of organ rejection.

POST-HEMILARYNGECTOMY ASSESSMENT

The dogs were assessed 2 months after hemilaryngeal reconstruction. Acoustic, aerodynamic, and endoscopic data were collected by the methods described above. After data collection, a laryngectomy was performed and the subjects were painlessly euthanized.

The larynges were fixed in formaldehyde and

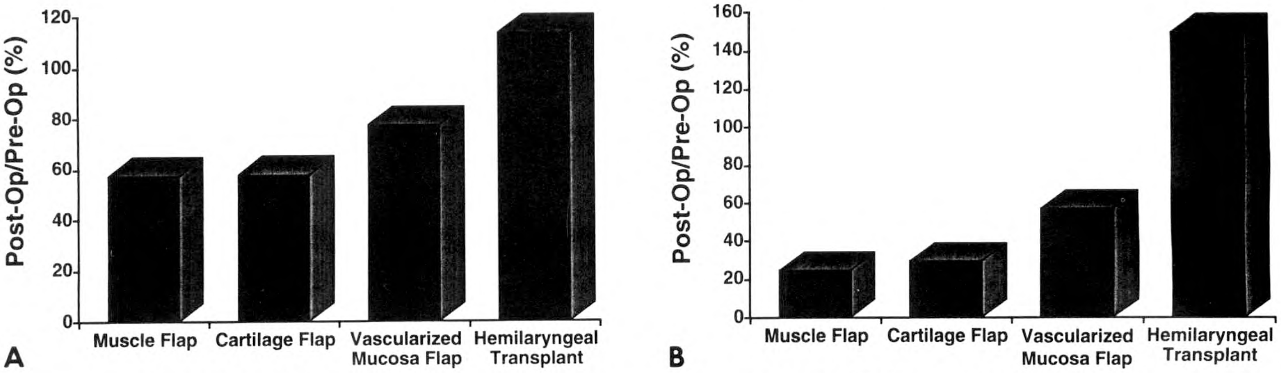

Fig 3. Hemilaryngeal reconstruction technique. A) Average acoustic power. B) Average aerodynamic power.

axially sectioned at the levels of the supraglottis, ventricle, glottis, and subglottis for gross and histologic examination. The specimens were then embedded in paraffin, and hematoxylin and eosin slides were prepared from each axial section.

RESULTS

CLINICAL OUTCOME

The study group included nine mongrel dogs for evaluation of autologous reconstruction techniques and one beagle that underwent hemilaryngeal transplantation. The second beagle was painlessly sacrificed after serving as the transplant organ donor.

Two of the mongrel dogs died in the postoperative period, both of airway obstruction from plugged tracheotomy tubes. The first was in the sternohyoid group and died during the third postoperative day. The other underwent buccal flap reconstruction and died on the eighth postoperative day. An autopsy indicated that neither aspiration, pulmonary infection, nor embolism was the cause of death. Replacement animals were substituted for these dogs, as described above.

The only additional complication was a postoperative seroma that did not respond to repeated aspiration and pressure dressings. The seroma resolved and the wound healed in approximately 10 days after drainage and wound irrigation. None of the subjects developed weight loss or clinical signs of aspiration.

ACOUSTIC AND AERODYNAMIC DATA

Some trial and error was required before consistent transtracheal stimulation of the RLN could be obtained. Therefore, the four preoperative and four postoperative trials with the highest calculated aerodynamic power were selected for each subject for further analysis. Figure 3A shows the average acoustic power for each condition, expressed as a percentage of the average preoperative values. Statistical significance could not be determined, because of the limited numbers of subjects and trials in each condition.

Figure 3B shows the aerodynamic power for each of the techniques, again expressed as a percentage of preoperative values. Aerodynamic power was highest for the hemitransplant dog; however, statistical

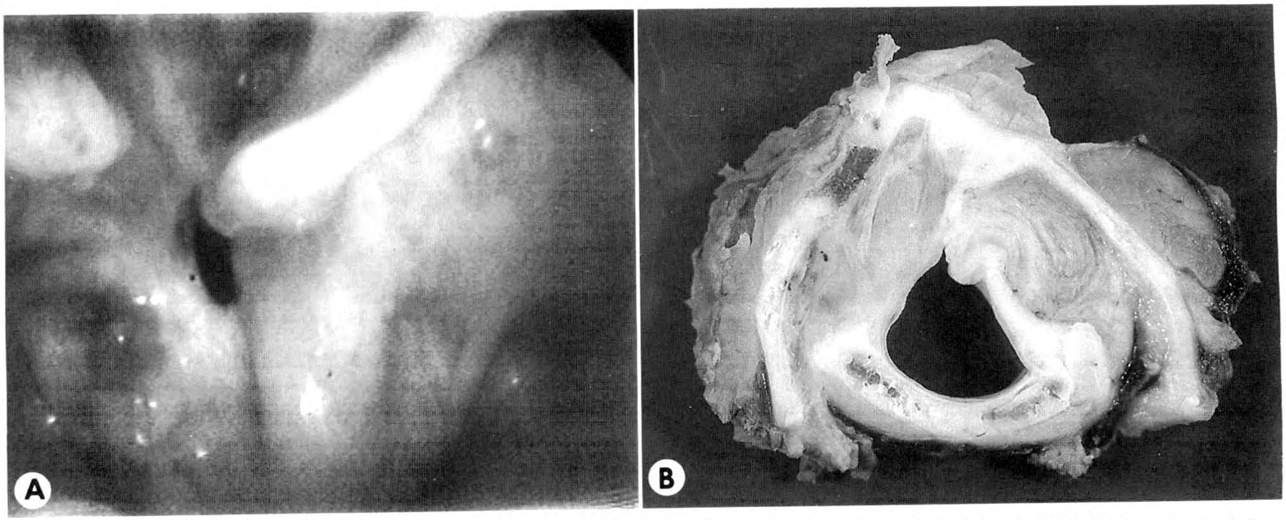

Fig 4. Left sternohyoid muscle flap, 2 months postoperative. A) Endoscopy with right RLN stimulation. B) Whole organ axial section of glottis.

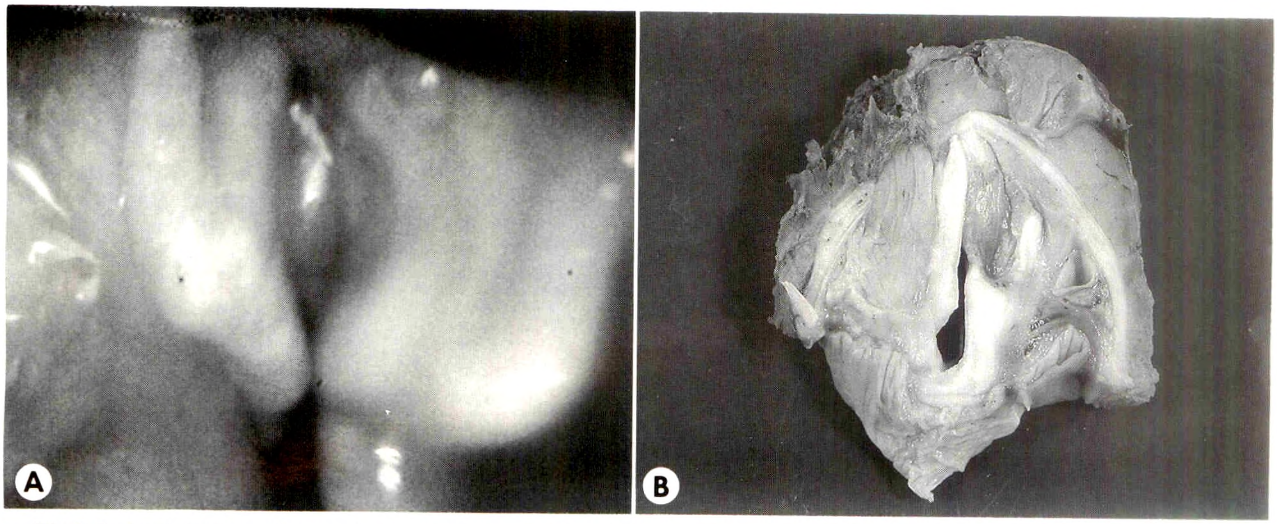

Fig 5. Left epiglottic cartilage and mucosa composite flap, 2 months postoperative. A) Endoscopy with right RLN stimulation. B) Whole organ axial section of glottis.

analysis was again limited by the small numbers of subjects and trials in each condition.

ENDOSCOPY AND WHOLE ORGAN SECTIONS

Sternohyoid Muscle Flap. Videoendoscopy of the superiorly based sternohyoid muscle flap demonstrated an adequate cross-sectional airway at the level of the glottis. A representative endoscopic photograph obtained during RLN stimulation is included as Fig 4A. The flap had mucosalized and the suture lines were well healed. The muscle flap (left) had atrophied posteriorly, resulting in a large posterior glottic gap that could not be closed even with overcompensation by the intact arytenoid. The anterior commissure was rotated to the left of the midline with a shortened anterior-posterior (AP) diameter of the glottis. The native vocal fold (right) was shortened and bowed even when the arytenoid was adducted upon RLN stimulation. This apparently resulted from the loss of support at the anterior commissure after resection of the anterior two thirds of the left thyroid cartilage.

The whole organ section (Fig 4B) confirmed the endoscopic findings of anterior commissure rotation and a shortened glottic AP diameter. The anterior edge of the thyroid cartilage was bent posteriorly, shortening the glottis and preventing the native vocal fold from tensing with adduction. The muscle flap had migrated anteriorly and atrophied posteriorly. There was a small amount of new cartilage and bone formation where the perichondrial flap was positioned external to the muscle flap. There was a thin layer of mucosa overlying the muscle flap on the endolaryngeal surface.

Epiglottic Cartilage Flap. Videoendoscopy of the epiglottic cartilage and mucosa composite flap demonstrated an adequate cross-sectional airway at the

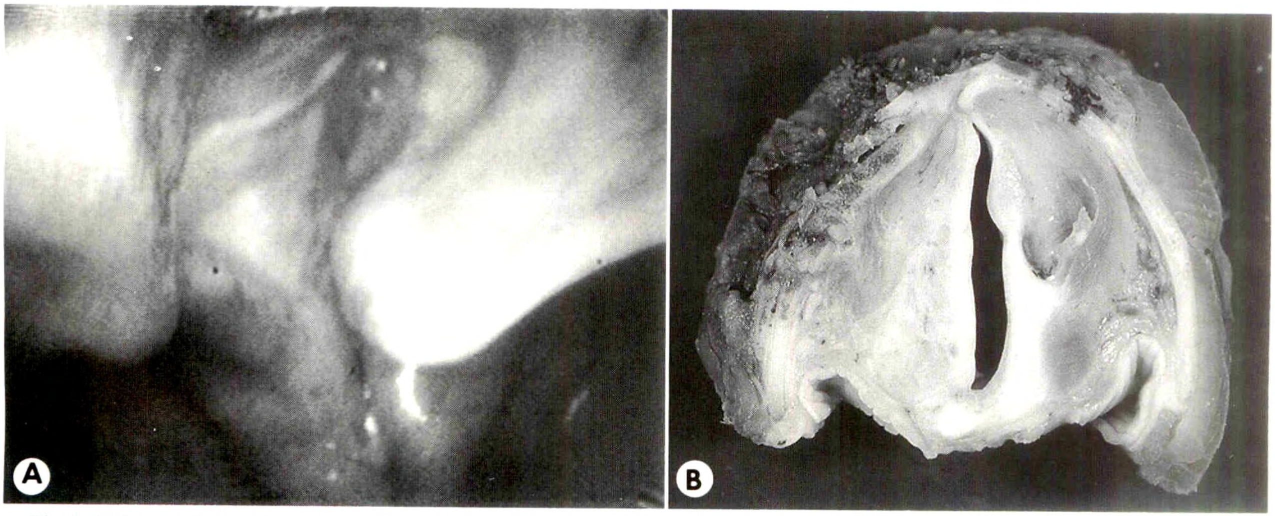

Fig 6. Left vascularized buccal mucosa and sternohyoid muscle flaps with microplate laryngeal framework reconstruction, 2 months postoperative. A) Endoscopy with right RLN stimulation. B) Whole organ axial section of glottis.

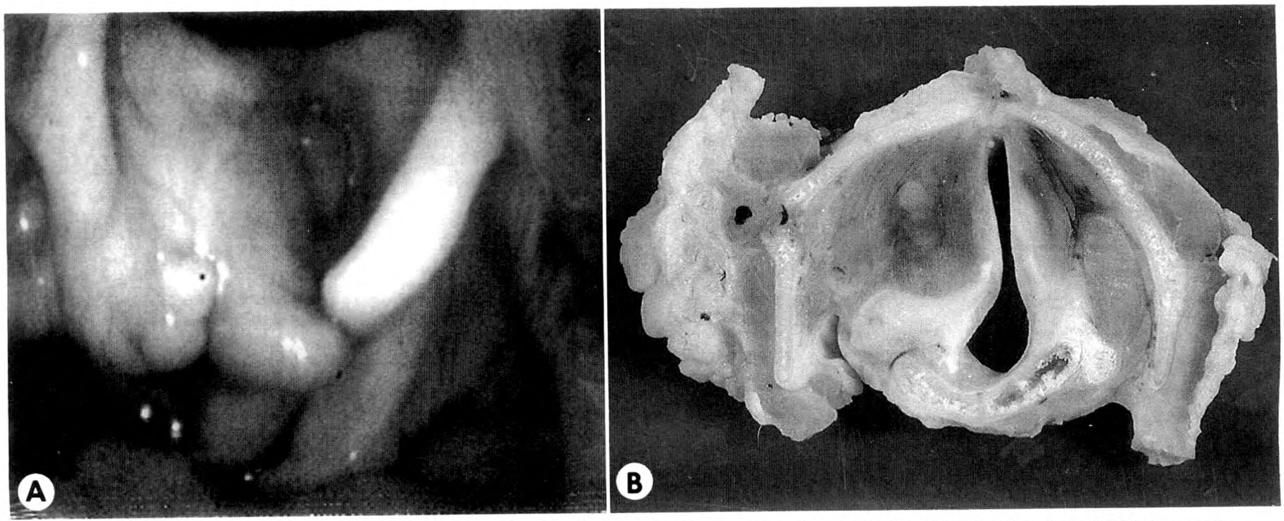

Fig 7. Left hemilaryngeal transplant with arytenoid adduction, 2 months postoperative. A) Endoscopy with right RLN stimulation. B) Whole organ axial section of glottis.

level of the glottis. A representative endoscopic photograph obtained during RLN stimulation is included as Fig 5A. The epiglottic mucosa on the endolaryngeal surface of the flap was intact and well vascularized. The posterior edge of the cartilage flap had pulled away from the cricoid in all three subjects, producing a large glottic gap. The remainder of the epiglottic flap was in the midline and met the contralateral vocal fold during RLN stimulation. There was a sharp anterior commissure where the epiglottic cartilage was sewn to the anterior thyroid cartilage, and a normal AP diameter of the glottis. There appeared to be some tension of the native vocal fold with RLN stimulation, perhaps due to cartilaginous support at the anterior commissure.

Whole organ sections (Fig 5B) confirmed the endoscopic findings of midline flap position, normal AP glottic diameter, and posterior glottic gap. There was little new cartilage or bone growth from the perichondrial flap.

Buccal Mucosa Flap. Videoendoscopy of the buccal mucosa flaps indicated an adequate cross-sectional airway at the level of the glottis. A representative endoscopic photograph obtained during RLN stimulation is included as Fig 6A. The vascularized mucosa flap (left) was fixed to the midline posteriorly. No glottic gap was present upon RLN stimulation. The anterior commissure was sharp and did not deviate from the midline. There was a normal AP diameter of the glottis. The native vocal fold adducted to the midline and tensed with RLN stimulation.

Findings on analysis of the whole organ sections (Fig 6B) were consistent with the endoscopic findings. The airway appeared small because of the position of the opposite vocal fold, but was adequate, with the native vocal fold abducted in the live animal. The miniplate had been incorporated into new cartilage and bone growth, apparently potentiated by the preserved overlying perichondrium.

Hemilaryngeal Transplantation. On endoscopic examination, the transplanted hemilarynx was similar in appearance to the native side (Fig 7A), although the fold was immobile and some edema was present. The native vocal fold abducted normally with respiration; the transplanted fold was fixed by an arytenoid adduction. The mucosa appeared to be healthy, without granulation or scarring. The vascularized mucosa flap (left) was fixed in the midline throughout the glottis and had no glottic gap during RLN stimulation. The anterior commissure was sharp and did not deviate from the midline. There was a normal AP diameter of the glottis. The native vocal fold adducted to the midline and tensed with RLN stimulation. At 2 months posttransplant, the transplanted vocal fold also demonstrated some tension with stimulation of the RLN.

On whole organ section, the airway appeared to be narrow (Fig 7B), but it was adequate clinically when the native vocal fold was abducted. Adduction of the transplant arytenoid was noted, with suture visible in the transverse section.

STROBOSCOPY

All dogs in the study underwent stroboscopic endoscopy. A mucosal traveling wave could not be elicited in any of the animals who underwent muscle or epiglottic flap reconstruction. The buccal flap and hemitransplant dogs revealed a traveling mucosal wave that was synchronous with the wave in the native vocal fold. In the buccal flap dogs, there was increased propagation of the mucosal wave superiorly to the supraglottis along the mucosal flap.

DISCUSSION

Previous studies of laryngeal reconstruction methods have emphasized issues of aspiration, voice, and airway. However, few authors have compared different reconstruction techniques by assessing phonation. Blaugrund et al[21] evaluated speech following reconstruction with mucosal and skin flaps. They found that phonation in these patients was primarily supraglottic, and that fundamental frequency was low and restricted in range. Preservation of the arytenoid improved both aerodynamic and acoustic parameters. Hirano et al[22] argued that such comparisons among different reconstruction techniques are problematic because functional results are not predictable, and because of a lack of standardized methods for evaluating vocal function.

This study evaluated four surgical techniques for reconstruction following vertical partial laryngectomy in the canine model. Preoperative and postoperative measures of vocal function were obtained to study the effects of each procedure on each individual subject. Aerodynamic power and acoustic power were measured because they are complementary indicators of vocal function. Aerodynamic power is a function of subglottic pressure, which in turn is a function of both glottic closure and tension. Acoustic power is related to both glottic closure and the periodicity of vibration, and reflects the ability of the larynx to produce acoustic intensity.

The canine larynx has provided insight into the physiology of the human larynx in normal and diseased states. However, several anatomic differences have implications for hemilaryngeal reconstruction. The arytenoid is longer in the dog and occupies about one half of the length of the posterior glottic gap, in comparison to one third in the human. The interarytenoid muscle in the dog is less developed and the posterior commissure closes less tightly during phonation.[23] Animals demonstrating a posterior glottic gap upon transtracheal RLN stimulation during preoperative phonation were excluded from the study. Following hemilaryngeal reconstruction, loss of tissue bulk could prevent the intact arytenoid from closing the posterior glottis, thus creating a glottal gap with a deleterious effect on postoperative phonation. Because the arytenoid is larger in the dog, the consequences of a posterior glottic gap during phonation may be greater than in humans.

Hirano[24] has emphasized the importance of the layered structure of the larynx. He described three anatomic and functional compartments of the larynx: the *cover*, consisting of the epithelium and the superficial layer of the lamina propria; the *transition*, consisting of the intermediate and deep layers of the lamina propria; and the *body*, formed by the vocalis muscle. During speech, the mucosa must move fluidly over the underlying muscle to produce the normal, wavelike motion at the glottis. After severe laryngeal trauma, for example, the mucosa and superficial lamina propria are scarred to the underlying muscle, producing nonvibrating segments of the vocal fold and aperiodic vibration.

For the reconstruction of hemilaryngeal defects, mucosal, muscle, and cartilage flaps have been most commonly used. Mucosal flaps alone do not adequately replace tissue bulk. When used as free grafts over muscle or cartilage, they generally undergo atrophy or necrosis. Muscle or cartilage flaps reconstitute tissue bulk, but when they are allowed to mucosalize, the normal vibratory properties of the glottis are lost, because the submucosa is thin and lacks the unique structural characteristics of the lamina propria. Following muscle or cartilage flap procedures, the hemilarynx usually vibrates poorly, as in the severely traumatized larynx. An ideal alternative for the endolaryngeal submucosa would have the viscoelastic properties of the native lamina propria.

A vascularized full thickness mucosal flap, such as the buccal flap used in this study, offers a theoretically acceptable autologous replacement for the laryngeal mucosa and lamina propria. The data in this study suggest that the viscoelastic properties of the buccal flap submucosa are more similar to those of the native lamina propria than either a mucosalized muscle flap or a cartilage and mucosa composite flap.

On stroboscopy, a mucosal wave occurred in the vascularized mucosal flap and hemilaryngeal transplant subjects. In subjects receiving muscle and cartilage flaps, no wave was identified and phonation was consistently aperiodic and perceptually rough. The lack of a mucosal wave is consistent with the clinical experience of many surgeons performing hemilaryngeal reconstruction in human patients.

The endoscopic data and whole organ axial sections provide anatomic evidence consistent with the phonation data in Fig 3. Both the muscle and cartilage techniques produced a posterior glottal gap. The muscle flap underwent atrophy and migration and the cartilage flap consistently pulled away from the posterior cricoid. This result detrimentally affected glottic closure and subglottic pressure, therefore reducing values for both acoustic and aerodynamic power. The muscle flap lacked framework reconstruction for the resected thyroid cartilage and therefore had no support for the anterior commissure. The results were a shortened glottic AP diameter and a native vocal fold that was unable to tense itself against the reconstructed hemilarynx. The cartilage flap provided some support of the anterior commissure. Unfortunately, in every dog the posterior edge of the epiglottic cartilage detached from the cricoid, producing a posterior glottic gap.

The vascularized buccal mucosa flap, combined with a muscle flap and laryngeal framework reconstruction, provided the best autologous repair of the excised tissue defect. This method replaced the thyroid cartilage support and the bulk of the intrinsic laryngeal musculature, resulting in both a normal AP glottic diameter and medialization of the entire neoglottis.

Hemilaryngeal transplantation was studied to investigate the results of an idealized autologous reconstruction. Although technically feasible, this is not an option for human partial laryngeal reconstruction at this time. Nonetheless, the dog that underwent hemilaryngeal transplantation achieved the best results in this study. Postoperative acoustic and aerodynamic power values were *better* than the preoperative baseline values, probably because of the tight glottic closure achieved by arytenoid adduction, which produced greater acoustic intensity and higher subglottic pressure.

In conclusion, three methods of autologous hemilaryngeal reconstruction and hemilaryngeal transplantation were compared in the dog. As expected, hemitransplantation produced the best acoustic, aerodynamic, and stroboscopic results, because it most closely replicated the layered laryngeal anatomy. The vascularized buccal mucosa flap also produced a favorable surface for laryngeal vibration, and functioned better than the cartilage and muscle flaps studied. Only the hemilaryngeal transplant and buccal mucosa flap produced a mucosal wave. The endoscopic and whole organ axial sections provide additional anatomic data that support the concept that when the excised hemilarynx is replaced by increasingly similar tissue, there is a successive improvement in vocal function.

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EXPRESSION OF MATRIX METALLOPROTEINASES AND TISSUE INHIBITOR OF METALLOPROTEINASES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes implicated in the invasion and metastasis of many cancers. In situ hybridization techniques were used to reveal sites of expression of collagenase (MMP-1), gelatinase 72 kd (MMP-2), gelatinase 92 kd (MMP-9), and tissue inhibitor of metalloproteinase-1 (TIMP-1) in head and neck carcinomas (N = 21). Both TIMP-1 and gelatinase 72 kd were expressed in nearly all tumors, whereas the expression of collagenase and gelatinase 92 kd showed variability. Tumor-associated expression of MMPs was strongest in stromal cells near advancing margins. No differences in expression levels were detected between primary and metastatic sites. This paper reviews the literature and discusses the significance and possible implications of MMPs in head and neck squamous cell carcinoma.

KEY WORDS — metalloproteinase, squamous cell carcinoma, TIMP, tissue inhibitor of metalloproteinase.

INTRODUCTION

It is well known that malignancies cause morbidity and mortality by local and distant destruction of their hosts' tissues. Head and neck cancers in particular are characterized by a high degree of aggressive growth and metastasis. Yet it has been only within the past several years that the mechanism of tumor invasion and metastasis has begun to be understood.

Tumor cell interaction with the basement membrane has historically been viewed as the crucial step in tumor invasion and future metastasis.[1] Basement membranes (composed of collagen type IV, laminin, and heparan sulfate proteoglycans, among other macromolecules) do not normally allow passive tumor cell migration to occur. Tumor invasion through the basement membrane is thought to be a three-step process. First, neoplastic keratinocytes attach to the basement membrane. Second, proteolysis of the basement membrane by enzymes secreted from malignant cells creates defects in the basement membrane. Third, tumor cells traverse the basement membrane and spread throughout adjacent connective tissue areas.

The initial steps of tumor invasion are likely repeated for further growth and metastasis of a tumor. The sequence of attachment, degradation, and migration just described for basement membrane invasion needs to be repeated within the extracellular matrix (ECM) for tumor growth to occur. One can extrapolate for metastases: tumor cells invade the basement membranes of blood vessels, travel through the bloodstream and attach to a distant organ, degrade basement membranes and ECMs, and migrate. Degradation of the ECM is without doubt a key element in tumor invasion.

The ECM-degrading proteinases (or proteases) produced by most invasive cells can be subdivided into three classes: 1) serine proteinases, 2) cysteine proteinases, and 3) matrix metalloproteinases (MMPs).[2] Serine proteinases include plasminogen activators (PAs), leukocyte elastase, and cathepsin G. The PAs have been the best characterized of the serine proteinases, and much evidence exists as to their involvement in cell invasion.[3,4] The PAs consist of two enzymes that are activators of plasminogen: urokinase-type PA (uPA) and tissue-type PA (tPA).

The cysteine proteinases are not as well characterized as the serine proteinases or the MMPs. They are

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mostly intracellular in location, and their role in tumor invasion is less certain.

The MMPs are a family of zinc metalloenzymes. They are secreted as proenzymes and require proteolytic cleavage to become activated. There are multiple members of the family, including 1) interstitial collagenase (MMP-1), 2) neutrophil collagenase, 3) stromelysin-1, 4) stromelysin-2, 5) stromelysin-3, 6) matrilysin, 7) gelatinase 72 kd (MMP-2), and 8) gelatinase 92 kd (MMP-9). The expression of MMP activity can be controlled at the level of gene transcription or proenzyme activation, or with the inhibition of enzymatic activity by specific tissue inhibitors. A multigene family of proteins named TIMPs (tissue inhibitors of metalloproteinases) has been demonstrated to inhibit fully activated MMPs. The local balance of these proteinases and proteinase inhibitors appears to be a crucial factor in tumor invasion and metastasis.

In this study, the spatial expression of a panel of MMPs, including MMP-1, gelatinase 72 kd (MMP-2), gelatinase 92 kd (MMP-3), and one inhibiting protein, TIMP-1, was evaluated by in situ hybridization techniques, and these data were analyzed within the context of the clinicopathologic features of head and neck squamous cell carcinoma.

METHOD

Tissue Preparation. Specimens from head and neck squamous cell carcinomas (N = 29) were collected at the time of surgical resection from a total of 21 patients at Vanderbilt University Medical Center. When possible, specimens were taken from three distinct areas: a peripheral margin of the primary tumor, the center of the primary tumor, and an area of bony invasion. Specimens were immediately fixed in ice-cold phosphate-buffered saline (PBS) 4% paraformaldehyde for 24 hours and embedded in paraffin. Sections (6 to 8 µm) were mounted on silanized glass slides for study.

In Situ Hybridization Technique. In situ hybridization was performed as described by Stricklin et al.[5] All sections were subjected to proteinase K digestion and acetylation. Prehybridization was performed for 3 hours at 42°C with a solution consisting of 50% formamide, 0.3 mol/L sodium chloride (NaCl), 0.02 mol/L Tris hydrochloride (Tris-HCl), pH 8.0, 0.005 mol/L ethylenediaminetetraacetic acid (EDTA), 1× Denhardt's solution, 10% dextran sulfate, and 0.01 mol/L dithiothreitol. Then, freshly labeled probe (20 µL and 1.2×10^6 cpm/100 µL prehybridization solution) was added directly to the prehybridization solution, and the slides were incubated at 50°C overnight in a chamber equilibrated with 4× standard sodium citrate (SSC) and 50% formamide in water. Hybridized sections were washed in 2× SSC, 10 mmol/L 2-mercaptoethanol, and 1 mmol/L EDTA (wash buffer) for 10 minutes at room temperature, then immersed in 20 µg/mL RNase A (Sigma Chemical Co, St Louis, Mo) in 0.5 mol/L NaCl, 0.01 mol/L Tris-HCl, pH 8.0, for 30 minutes at room temperature. After two washes at room temperature, the sections were sequentially rinsed for 5, 20, and 95 minutes at 55°C in wash buffer modified to contain 0.1× SCC. After two additional washes at room temperature in 0.5× SSC, the sections were dehydrated in a graded series of ethanol rinses containing 0.3 mol/L ammonium acetate. The slides were dried and dipped in NTB-2 photographic emulsion (Kodak) and incubated at 4°C for 2 weeks. After development, toluidine blue was used as a counterstain to aid in visualization of the tumor morphology. Slides were examined under both bright- and dark-field conditions. All tissues were hybridized with both sense and antisense riboprobes to establish the specificity of the method.

Probes. Four different riboprobes were used to examine which cells were expressing MMPs. Interstitial MMP-1 was obtained from the American Type Culture Collection (Rockville, Md), and a 763 base pair segment was amplified by polymerase chain reaction. We obtained TIMP complementary DNA, an 810 base pair fragment, from Synergen, Inc, Boulder, Colo.[5] For MMP-2, a 569 base pair fragment was prepared from the carboxy-terminal coding region as previously described.[6] A 343 nt sequence was amplified from a complementary DNA clone MMP-9 obtained from Dr Margaret Hibbs, Veterans Administration Medical Center (Newington, Conn). The primer pair was 5'-TGG GCT TAG ATC ATT CCT CAG TGC and 5'-AAG ATG TTC ACG TTG CAG GCA TCG. Computerized searches were used to avoid regions of high similarity with other MMPs (IBI Pustell), and primer pairs were developed with the Primer Detective software. The amplified DNA was directly inserted into a plasmid (TA Cloning Kit, Invitrogen), excised with EcoR1, and ligated into pGEM3Z vector (Promega) for production of sense and antisense riboprobes.

Classification of Samples. A modification of the Autio-Harmainen et al[7] classification was used to classify our samples into one of four groups. Group (–) had signal indistinguishable from the background; group (±) had a slight increase in signal compared with background labeling (<5% of cells); group (+) had easily identified areas of increased signal intensity (5% to 50% of cells involved); and group (++) had a strong response, with greater than 50% of cells being labeled.

TABLE 1. CORRELATION OF SITE, STAGE, AND RECURRENCE WITH MMPs AND TIMP-1 IN SQUAMOUS CELL CARCINOMA

Case	*Age*	*Site*	*Stage*	*Follow-up (mo)*	*Recurrence*	*MMP-1*	*TIMP-1*	*MMP-2*	*MMP-9*
1	59	Supraglottis	T1N3	2	Yes	−	+	+	++
2	42	Supraglottis	T4N1	15	No	+	+	+	++
3	71	Supraglottis	T2N0	5	Yes	++	+	+	±
4	50	Supraglottis	T4N3	8		−	+	−	−
5	73	Oropharynx	T3N3	16	No	±	+	+	+
6	55	Oropharynx	XRT	5	Yes	±	+	+	±
7	57	Oropharynx	T1N3	9	Yes	−	++	+	−
8	79	Oropharynx	T4N2c	14	Yes	++	++	+	−
9	46	Oropharynx	T4N0		No	++	++	++	−
10	65	Oropharynx	T4N0	8	No	++	+	++	−
11	68	Oropharynx	T3N1	4	No	−	+	−	−
12	65	Hypopharynx	T4N0	15	No	±	++	±	−
13	68	Hypopharynx	T2N0	15	No		+	±	±
14	63	Hypopharynx	T3N2b	4	Yes	++	++	+	±
15	62	Larynx	XRT	18	No	+	++	+	−
16		Larynx	T3N3		No	+	++	+	±
17	73	Oral cavity	T4N0	15	Yes	±	++	±	±
18	47	Oral cavity	T2N0	20	No	+	±	+	−
19		Oral cavity	T4N2b	10	Yes	±	+	++	−
20	81	Oral cavity	T2N0		No	+	+	±	−
21	60	Maxillary sinus	T4N0	4	No	+	+	++	++

MMP — matrix metalloproteinase, TIMP-1 — tissue inhibitor of metalloproteinase, XRT — radiotherapy, − — signal indistinguishable from background, ± — slight increase in signal compared with background (<5% of cells), + — 5% to 50% of cells labeled, ++ — >50% of cells labeled.

RESULTS

A total of 21 primary tumors were collected and subjected to in situ hybridization with MMP-1, MMP-2, MMP-9, and TIMP-1 probes. In accordance with the TNM staging system, transcripts for each MMP and TIMP-1 were compared to the tumor size, nodal status, and overall stage (I through IV). Two specimens were obtained from patients who had failed radiotherapy. Since these tumors were therefore not stageable, they were not included in the staging analysis.

In addition, 6 tumors were large enough that the tumor center and the tumor margin were clearly distinct from each other. This allowed these 6 centers and margins to be analyzed separately. Last, 2 of the primary tumors that had progressed to bony invasion were included in the analysis. For an overview of the data, see Table 1.

Expression of MMP-1 in Primary Tumors. Significant levels of MMP-1 expression were found in 12 of 21 (57%) primary tumors. Signals were present mostly within the stromal cells surrounding nests of tumor cells, but transcripts were also detected within tumor cells (Fig 1A,B). The MMP-1 was expressed in 0 of 2 T1, 4 of 4 T2, 1 of 4 T3, and 6 of 9 T4 cancers. Correlation of collagenase expression in the primary tumor in the presence or absence of nodal involvement revealed transcripts in 7 of 9 N0 and in 4 of 10 N1-3 tumors. Transcripts for MMP-1 were present in 4 of 4 stage II and 7 of 15 stage IV cancers.

Expression of MMP-2 and MMP-9 in Primary Tumors. We found MMP-2 in 14 of 21 (67%) primary tumors, and MMP-9 in 5 of 21 (24%) of the tumors. The MMP-9 was expressed in a larger proportion of tumor cells (when present) compared to MMP-2, which was mostly confined to the stromal cells and endothelial cells (Figs 1C,D and 2A,B). The MMP-2 was expressed in 2 of 2 T1, 2 of 4 T2, 2 of 4 T3, and 6 of 9 T4 cancers, and in 5 of 9 N0 and 7 of 10 N1-3 tumors. The MMP-9 was present in 2 of 4 stage II and 10 of 15 stage IV cancers. Labeling for MMP-9 was found in 1 of 2 T1, 1 of 4 T2, 1 of 4 T3, and 2 of 9 T4 lesions. Two of 9 N0 and 3 of 10 N1-3 cancers had MMP-9 expression, as did 1 of 4 stage II and 4 of 15 stage IV cancers.

Expression of TIMP-1 in Primary Tumors. TIMP-1 was detected in significant levels in 20 of 21 (95%) primary tumors. Prominent labeling was confined to the stroma surrounding tumor cells and within endothelial cells (Fig 2C,D). We found TIMP-1 expressed in 2 of 2 T1, 3 of 4 T2, 4 of 4 T3, and 9 of 9 T4 lesions and in 8 of 9 N0 and 10 of 10 N1-3 cancers. It was expressed in 3 of 4 stage II and 15 of 15 stage IV

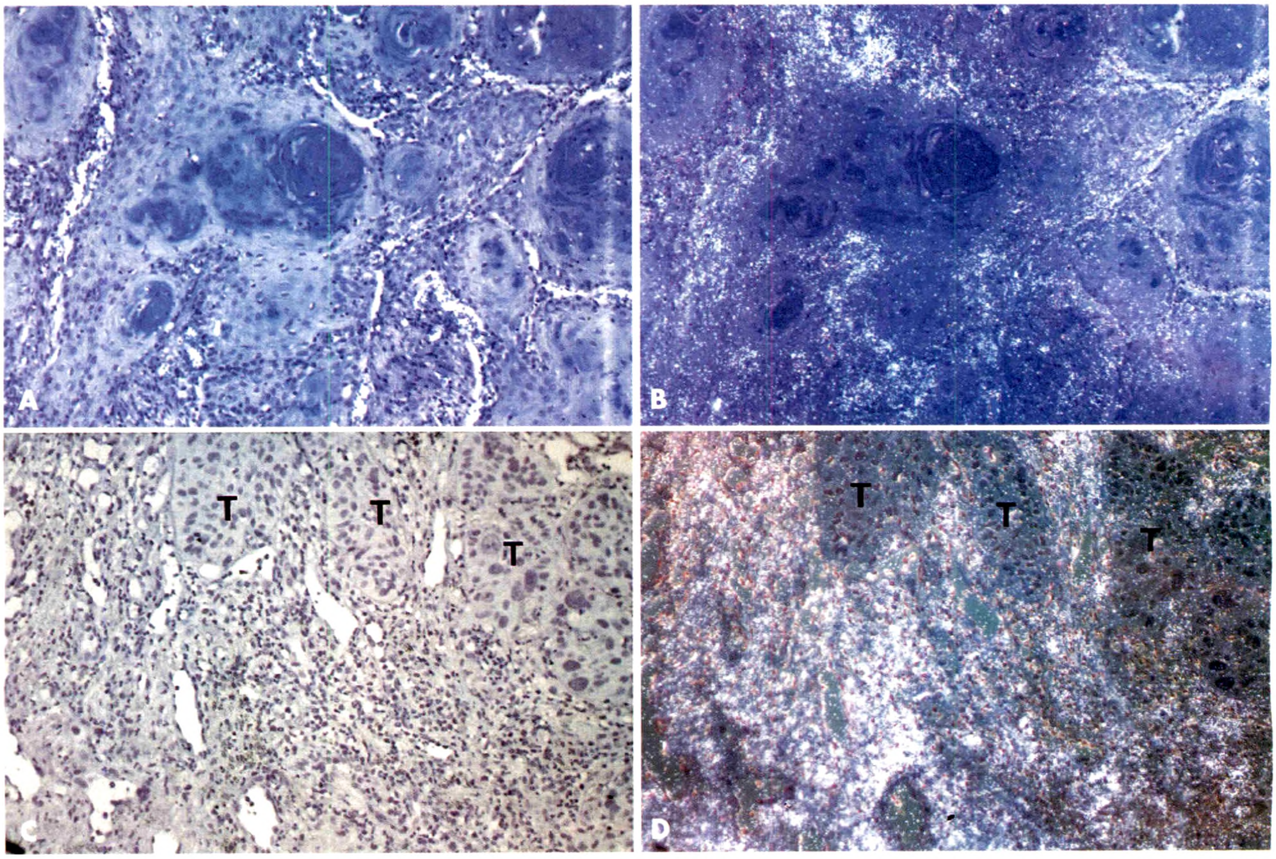

Fig 1. A,B) Bright-field (A) and dark-field (B) views (original ×165) of T4N0 well-differentiated squamous cell carcinoma (SCCA) of oropharynx demonstrate intense expression of transcripts for matrix metalloproteinase (MMP)–1 (collagenase) in inflammatory component of stroma. No expression is present either in tumor margin or within keratin pearls. C,D) Matching bright-field (C) and dark-field (D) photomicrographs (original ×250) of MMP-2 (gelatinase 72 kd) in T3N3 SCCA of oropharynx. Transcripts for MMP-2 are present throughout tumor-associated stroma and are conspicuously absent within tumor (T) itself.

cancers.

Expression of MMP-1, MMP-2, MMP-9, and TIMP-1 in Bony Invasion. Two tumors with bony invasion were examined. In both cases, MMP-1 and TIMP-1 expression were detected at the tumor-bony interface (Fig 3). Neither MMP-2 nor MMP-9 demonstrated expression in any areas of bony-tumor interface.

Relationship Between Expression of MMP-1, MMP-2, MMP-9, and TIMP-1 and Clinical Stage. For an overview of the clinicopathologic correlations, see Table 2. There were no statistically significant relationships between the number of samples with positive expression and the T status, N status, or stage for any of the MMPs or TIMP-1.

Relationship Between Expression of MMP-1, MMP-2, MMP-9, and TIMP-1 and Clinical Outcome. The average length of follow-up was 10.3 months. During that period of time, 6 of 19 patients had recurrence of their tumor, with an average follow-up of 15 months for those with recurrences and 9 months for the 13 that were free of disease. The MMP-1 was expressed in 2 of 6 patients with recurrences and 9 of 13 patients free of disease. We found MMP-2 in 5 of 6 patients with recurrences and 7 of 13 patients free of disease. One of 6 patients with recurrences and 4 of 13 patients free of disease had MMP-9 expression. We found TIMP-1 expressed in all patients except for 1, who was without recurrence. No statistically significant correlations were found when comparing recurrent disease to the presence or amount of any given MMP or TIMP-1.

Expression of MMPs and TIMP-1 in Tumor Center and Tumor Margin. Six tumors were large enough to allow a section to be sampled from the center and compared to a section taken from the margin. In situ hybridization revealed no statistically significant differences or trends between each messenger RNA analyzed and the location of the specimen within the tumor.

Comparison of Expression of MMP-1, MMP-2, MMP-9, and TIMP-1 in Each Tumor. To evaluate whether increased expression for MMPs was a general response commonly found in most squamous cell carcinomas originating in the head and neck region, the three MMPs and TIMP-1 were compared

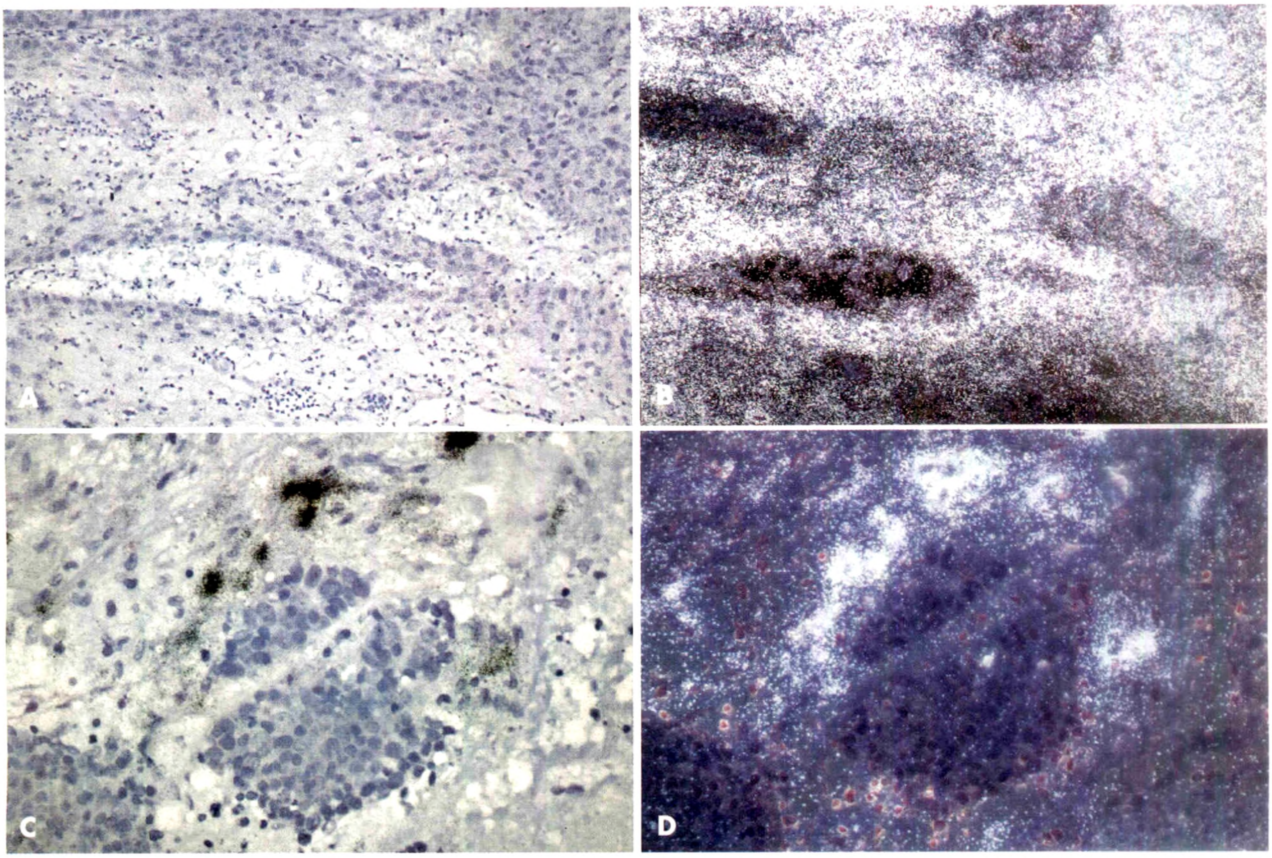

Fig 2. A,B) Matching bright-field (A) and dark-field (B) photomicrographs of MMP-9 (gelatinase 92 kd) in T4N1 SCCA of supraglottis (original ×200). Intense labeling is present in both tumor cells and surrounding stroma. C,D) Matching bright-field (C) and dark-field (D) photomicrographs of tissue inhibitor of metalloproteinase (TIMP)–1 in T1N3 SCCA of supraglottis (original ×500). Expression of transcripts for TIMP-1 is focally intense within some of inflammatory cells surrounding nest of tumor cells.

and contrasted within each individual tumor. No correlations were observed among the expression of MMP transcripts within individual tumors. These findings indicate that for at least three differing MMPs, there is not merely a constitutive elevation in squamous cell carcinoma. The presence of abundant transcripts for TIMP-1 in the presence of increased levels of MMPs within individual tumors suggests that the role of MMPs in tumors is not limited to tumor invasion, but involves a complex equilibrium between the amount expressed of any enzyme and that of any other enzyme.

DISCUSSION

Although MMPs are commonly expressed in tumor-associated stroma and occasionally in tumor itself, the full details of the contribution to invasion and metastasis are not known. A number of studies have tried to delineate which, if any, of these proteinases are required for various tumors to grow and spread and which, if any, may serve as prognostic indicators. In our study, in situ hybridization was selected as the preferred technique, since it would indicate which cells were actively expressing MMPs. In our previous study that examined the immunodistribution of MMP proteins, these matrix proteins were no longer associated with cells, but were diffusely present within the extracellular space, where they are actively involved in proteolytic remodeling of the tissue.[8]

Some of the results of our study are compatible with studies previously performed as described. Previously described histologic descriptions of cells that were stained positive for the expression of the various proteinases by in situ hybridization techniques were similar, if not identical, to our results. The most heavily stained regions in a given tumor involved the stromal elements (mostly fibroblasts) surrounding nests of tumor cells and within endothelial cells. It has been hypothesized that tumor cells secrete some paracrine substance that induces surrounding stromal cells to produce these proteinases. Few, if any, tumor cells actively expressed TIMP-1 or MMP-1, although MMP-2 and MMP-9 were expressed, sometimes heavily, by tumor cell nests.

Our attempts to correlate expression of one or a combination of MMPs with tumor behavior or clini-

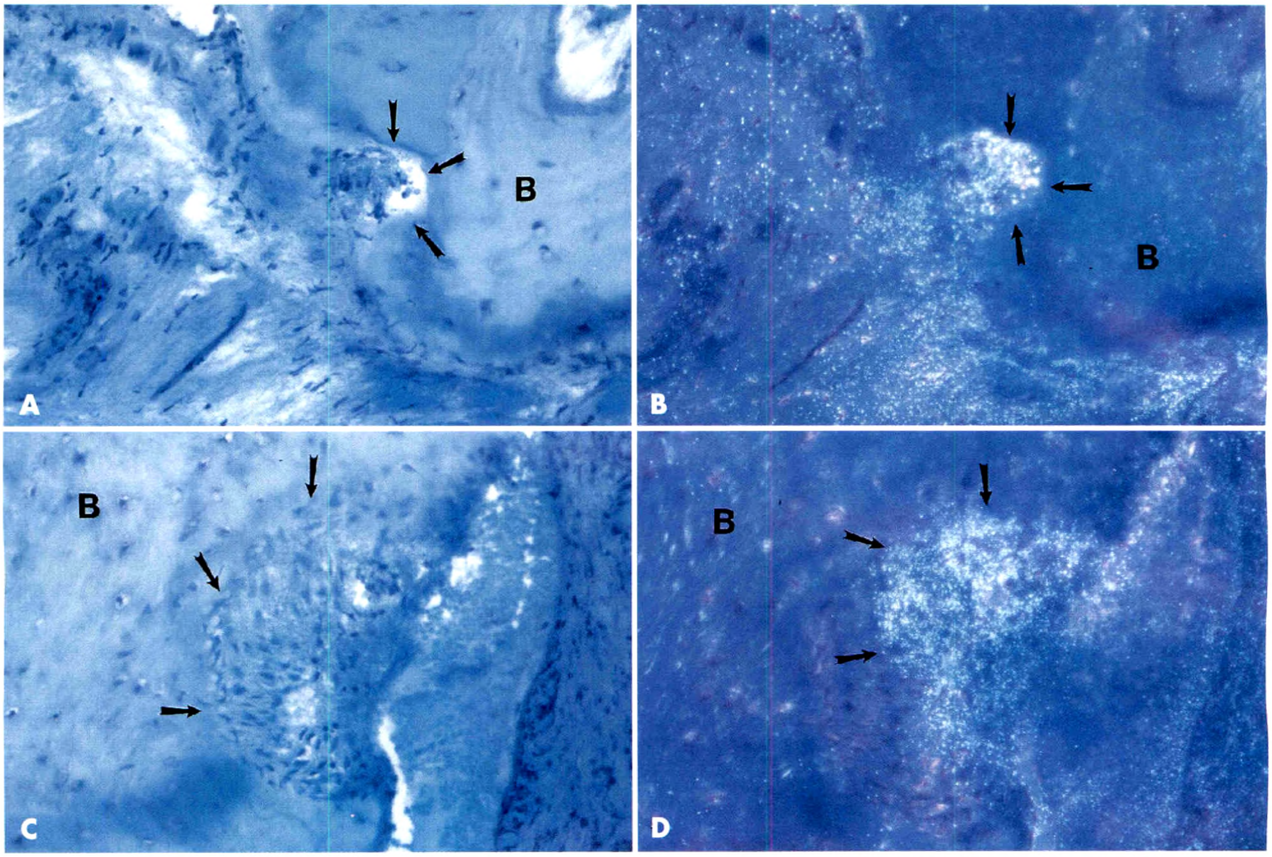

Fig 3. Matching bright-field (A) and dark-field (B) photomicrographs of MMP-1 in T4N2c SCCA of oropharynx (original ×300). Note pocket of invading carcinoma (arrows) in bone, with intense expression of MMP-1 focally at site of invasion. Little or no activity is detected in nonadjacent stroma or bone. C,D) Matching bright-field (C) and dark-field (D) photomicrographs of TIMP-1 in same tumor as in A and B (original ×330). Note nest of tumor cells invading bone, with expression of TIMP-1 transcripts.

cal staging did not reveal any significant associations. This differs somewhat from some of the previous reports described below, which demonstrated statistically significant correlations with markers such as tumor invasiveness and lymph node metastases. Stage IV carcinomas made up the majority of our sample, and our conclusions may have been shaped by the fact that our specimens represent the most aggressive tumors. However, we were unable to demonstrate any correlation between proteinase activity in the primary tumor and a propensity for the tumor to spread to regional lymph nodes. Also, our attempt (albeit with a small sample) to correlate proteinase activity of a metastatic node with the proteinase activity of the primary was not successful. Given our results, and given the fact that only one study has suggested a correlation between a clinically significant prognostic indicator and proteinase activity, we believe that other, more significant factors are involved in tumor invasion and metastatic capabilities. Much has yet to be explored in this area of proteinase activity and cancer.

Bony invasion is an area that has not been addressed in other reports on this subject. Difficulty in obtaining specimens with this feature, along with increased technical difficulty in performing in situ hybridization on these samples, makes bony invasion hard to investigate. Our sample of 2 makes it impossible to attempt any correlations between the type or amount of proteinases involved and bony infiltration. However, our finding of MMP-1 and TIMP-1 at the tumor-bony interface suggests a possible relationship between bony invasion and MMP activity.

We hypothesized prior to this study that the tumor margins would demonstrate increased proteinase activity to facilitate tumor invasion compared to the tumor center, which is not directly involved in this process. However, we found no differences in the amount or type of proteinase secreted between tumor centers and margins. It is now our belief that the entire tumor may nonselectively cause secretion of these factors, rather than just the tumor's pushing margins using these enzymes to facilitate invasion of adjacent tissue. Expression of MMP appears to be a property of the whole tumor, and not just a reaction of the advancing tumor to the host's tissues. We acknowledge that our sample for this group is small (n = 6) and that more investigations are needed to provide further

TABLE 2. CLINICOPATHOLOGIC CORRELATIONS OF MMPs AND TIMP-1 WITH SQUAMOUS CELL CARCINOMA

	MMP-1	*TIMP-1*	*MMP-2*	*MMP-9*
T stage				
T1	0/2	2/2	2/2	1/2
T2	4/4	3/4	2/4	1/4
T3	1/4	4/4	2/4	1/4
T4	6/9	9/9	6/9	2/9
Nodal status				
N0	7/9	8/9	5/9	2/9
N1-3	4/10	10/10	7/10	3/10
Stage				
I				
II	4/4	3/4	2/4	1/4
III				
IV	7/15	15/15	10/15	4/15

Data are numbers of patients.

MMP — matrix metalloproteinase, TIMP-1 — tissue inhibitor of metalloproteinase.

proof of this hypothesis.

Only a handful of investigations have examined the role of these MMPs in head and neck squamous cell carcinoma. Kusukawa et al[9] looked at gelatinase 72 kd (MMP-2) in oral cancer and found that its presence in 31 of 46 (67%) patients correlated with lymph node metastases. In their series, MMP-2 was strongly expressed in 77% of patients with lymph node metastases, whereas it was detected in only 25% of patients without nodal metastases. However, an evaluation of the relationship between MMP-2 and either clinical stage or histologic differentiation did not demonstrate statistical significance.

Polette et al[10] localized TIMP-1 in 18 of 18 squamous cell carcinomas and TIMP-2 in 13 of 18 squamous cell carcinomas. They found that the presence of TIMP-1 and TIMP-2 suggested a less aggressive tumor. The same authors also demonstrated collagenase I (MMP-1) and stromelysin-2 in 23 of 26 head and neck tumors. These MMPs were found principally within fibroblasts surrounding the tumor, and in endothelial cells of vascular structures, but not within the tumor cells.[11] It has been suggested that the TIMPs may be protecting the integrity of the vascular basement membrane, and TIMP-1 has been demonstrated to limit tumor cell invasion of human amnion in vitro and to prevent metastasis formation in animal models.[12,13]

When the expression of MMP-2 and MMP-9 was evaluated in 27 hypopharyngeal squamous cell carcinomas, expression of MMP-2 and MMP-9 was found in 78% and 85% of tumors, respectively.[14] In that study, no correlation was noted between the expression of either proteinase in the primary tumor with histologic classification, clinical stage, lymphatic spread, or presence of extracapsular spread. However, the investigators did find a statistical correlation between outcome of treatment and MMP-2 expression and stated that the presence of MMP-2 was indicative of a poor outcome.

Several investigators have evaluated MMP-1 production in head and neck squamous cell carcinomas. Muller et al[15] found high levels of MMP-1 expression in 80% of tumors and did not detect expression in normal tissue. There was not a significant correlation found between the levels of expression of the primary and lymph node metastases. Polette et al[11] detected MMP-1 expression in 18 of 26 head and neck carcinomas. The expression of MMP-1, when detected, was always localized in the stromal cells, but additional expression was found in small tumor cell nests in 4 of those cases.

Stromelysin is another MMP that has been shown to have high expression in head and neck squamous cell carcinomas. Muller et al[16] positively correlated stromelysin-3 gene expression with increased local invasiveness in head and neck squamous cell carcinomas. They identified stromelysin-3 gene expression in 106 of 111 (95%) primary and 19 of 21 (90%) metastatic head and neck squamous cell carcinomas. The expression was detected in stromal fibroblasts immediately surrounding tumor cells, but not in fibroblasts within normal mucosa sampled at least 5 cm from the tumor site. The pattern and degree of expression were similar to those seen in breast carcinoma. No statistically significant differences were found when comparing the levels of stromelysin expression and either the tumor size, lymph node involvement, or extracapsular lymph node invasion. Correlations were made, however, when comparing stromelysin-2 levels to tumor invasiveness, and the authors suggested that messenger RNA levels of stromelysin may be a useful indicator for predicting the clinical aggressiveness of these tumors. Another stromelysin, stromelysin-3, has been found to be expressed in the stromal cells of several tumors, including head and neck cancer. Of interest, it is the only MMP gene that has been found to be regularly overexpressed in invasive breast carcinomas without being overexpressed in benign lesions.[17]

SUMMARY

The exact role the MMPs and proteinase inhibitors play in tumor invasion and metastasis is still largely speculative. Despite the consistent observations that these proteins are excessively expressed in multiple tumor types, it is unknown which, if any, are required

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for invasion and metastasis. Whether the MMPs function independently of each other or whether some yet-unknown factor is responsible for the coordination of the expression of these proteinases requires further investigation. The only consistent finding in our study that also pervades other studies of squamous cell carcinoma of the head and neck is the presence of TIMP-1 overexpression in almost every tumor. Whether this phenomenon represents an attempt by the host to inhibit the other MMPs or whether TIMP-1 may itself be a mechanism of tumor invasion needs to be addressed.

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ACOUSTIC MEASUREMENT OF CHANGE IN VOICE QUALITY WITH TREATMENT FOR CHRONIC POSTERIOR LARYNGITIS

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Sixteen patients who had symptoms and signs of chronic posterior laryngitis were evaluated before, during, and after treatment with omeprazole and nocturnal antireflux precautions. Data were analyzed for patients who complained of some hoarseness, who had no smoking history, and who completed all of the voice recording protocol. The patients' voices were recorded before, during, and following treatment with omeprazole and nocturnal antireflux precautions. Voice quality was analyzed by perceptual analysis, and acoustic signal data were measured for jitter, shimmer, and signal-to-noise ratio. Measures of jitter, shimmer, and signal-to-noise ratio changed significantly with treatment of posterior laryngitis ($p < .01$ for change in each of the measures). Acoustic measures showed some trend of deterioration with cessation of treatment, although the overall improvement in acoustic measures of voice quality was still statistically significant after treatment with omeprazole was discontinued. Although perceived abnormality of voice increased and decreased with the magnitude of measured perturbation of the acoustic signal for some patients, the perceptual assessments were not highly correlated with acoustic measures for individual patients, and the perceptual analysis group data did not show a significant change with time during treatment, in contrast to the significance of change in acoustic measures. The data demonstrate that acoustic measures of jitter, shimmer, and signal-to-noise ratio improve significantly with antisecretory and antireflux treatment of chronic posterior laryngitis, and that for individual patients, these are changes that are detected by trained listeners, but not at statistically high levels of confidence.

KEY WORDS — acoustic measures, laryngitis, voice.

BACKGROUND

Many patients with chronic laryngitis complain of recurring hoarseness as a bothersome part of their symptoms. In addition to voice changes, these patients complain of recurring or chronic sore throat, postnasal drip, throat clearing, chronic cough, and globus symptoms.[1] The symptom of recurring hoarseness is more likely to be a significant problem for patients who professionally require substantial voice use. These individuals typically complain that voice quality deteriorates with voice use during the day and recovers with voice rest. Although this vocal dysfunction may be a problem for the patient who uses his or her voice professionally, the degree of dysphonia may or may not be perceptually pathologic to the laryngologist or speech pathologist at the time of an examination. Changes in relatively subtle dysphonia may be even more difficult to appreciate over time by the usual subjective clinical assessment.

This project was part of a larger study of the efficacy of antisecretory and antireflux treatment for symptoms of chronic laryngitis. The laryngeal complaints of the patients, as represented by symptom index, responded well to treatment. Figure 1 shows symptom index data that have been reported.[2] Laryngeal symptoms responded dramatically to antisecretory and antireflux treatment. This part of the study addressed the hypothesis that for patients who complain of hoarseness in association with other symptoms of chronic laryngitis, treatment of the laryngitis will be associated with a change (improvement) in objective acoustic perturbation measures of voice quality. Therefore, this study examined acoustic perturbation measures of voice quality over time in patients who complained of hoarseness along with other symptoms and signs of chronic laryngitis and who were treated with nocturnal antireflux precautions and omeprazole, a potent suppressor of gastric acid secretion.[3]

METHODS

Subjects. The subject population for this study was selected from consecutive patients who presented to a multispecialty group practice complaining of chronic symptoms relating to their throat or voice. Chronic symptoms were defined as those present longer than 3 months. These symptoms included recurring sore or irritated throat, chronic or recurring hoarseness, and chronic cough in association with soreness and/or hoarseness. On examination, all of these patients

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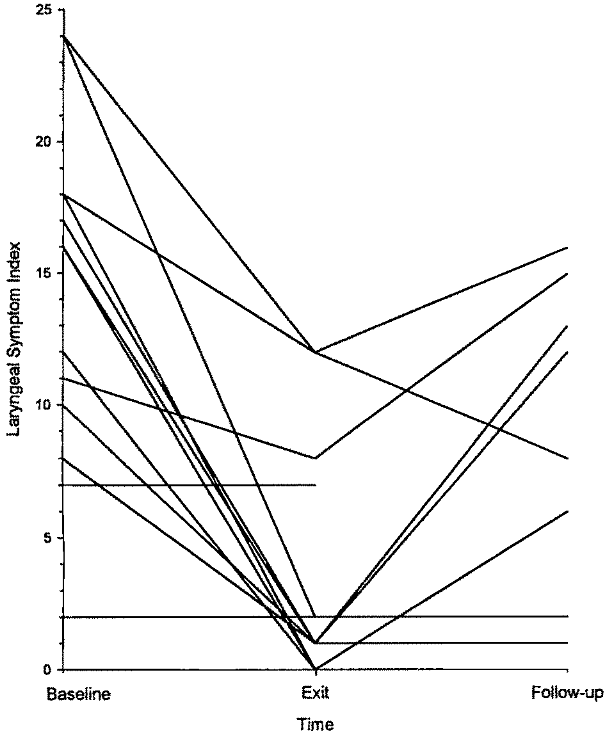


Fig 1. Laryngeal symptom indices reflect frequency and severity of symptoms of cough, nocturnal cough, throat clearing, throat burning, and hoarseness. Kamel et al[2] reported that with antisecretory and antireflux treatment, laryngeal symptom indices changed from 13.94 ± 1.50 to 3.0 ± 1.6 at exit ($p < .05$), but that there was exacerbation of symptoms within 6 weeks of stopping antisecretory treatment.

had visible signs of inflammation of the laryngeal structures that included variable degrees of erythema, hypervascularity, granular thickening of the epithelium, edema, and stasis of secretions. From that group, patients who had symptoms and signs of chronic irritative laryngitis, and who agreed to participate in a prospective study to assess the efficacy of gastric acid suppression for treatment of chronic irritative laryngitis, were entered into a prospective protocol. Patients had a baseline assessment of symptoms and underwent video-documented laryngoscopy and esophagoscopy. Symptom index, laryngoscopic examination, and voice recordings were assessed at 6-week intervals during treatment. The data on resolution of laryngitis symptoms (Fig 1) and data on improvement of signs of laryngeal inflammation with acid suppression have been reported.[2] Patients in that study who presented with a complaint of hoarseness, who had never smoked, and who completed the voice recording protocol of the prospective treatment outcome study were included in this analysis. Of 16 patients who completed a detailed prospective treatment outcome study protocol for chronic laryngitis, 6 had a history of smoking or did not complain of hoarseness as a presenting symptom. Voice recordings that were usable for measurement were not available from each treatment visit, for various reasons, for 4 patients. Therefore, voice recording data were available for 6 patients with hoarseness and no smoking history. These 6 patients varied in the degree of vocal abnormality that was perceptually apparent at the start of the study.

Treatment Protocol. Patients with chronic laryngitis symptoms were treated with antireflux precautions and omeprazole 20 mg daily. When patients achieved resolution of laryngitis symptoms, omeprazole was discontinued and the patients were advised to continue with nocturnal antireflux precautions. The patients were followed up at approximately 6-week intervals. Most patients experienced symptom resolution by 6 to 9 weeks and discontinued omeprazole at that time. One patient did not achieve resolution of symptoms or signs of laryngitis during the study. That patient subsequently had improvement on high doses (80 mg daily) of omeprazole.

Voice Recording. Voice recordings were done at entry, at 3 to 4 weeks, at 6 to 9 weeks, at 12 to 15 weeks, and at 18 to 28 weeks. For each recording, the patient was seated in a 2.4 × 2.4-m^2 (8 × 8-ft) sound-treated room. Three samples of sustained phonation of the vowel /a/ were recorded on an Ampex AG350-2 reel-to-reel tape recorder through an Electro-Voice 664 Dynamic Cardioid microphone.

Analysis. The acoustic recordings were subjected to both perceptual and acoustic signal analyses. A 5-second segment of sustained vowel phonation for each patient at each recording session was used as the stimulus for a perceptual task. Three trained listeners served as subjects; each listener was a speech-language pathologist with at least 1 year of experience either treating or analyzing dysphonic voices. All listeners had normal hearing and demonstrated no speech, voice, or language disorders themselves.

The measurement scale for the listening task consisted of an undifferentiated line scale with labeled anchors. This type of scale is known as a visual or linear analog scale and is used in psychophysiologic research to evaluate qualitative stimuli.[4-6]

Each listener was told that he or she would hear a series of speakers phonating the vowel /a/. After listening to each sample, the listener was asked to rate the voice on the scale, from totally unacceptable to totally acceptable, by placing a slash mark along the visual analog scale where they felt it was appropriate. For scoring purposes, the visual analog scale was converted to a 100-point rating scale in which a score of 0 indicated a totally unacceptable voice and a score of 100 indicated a totally acceptable voice. The mean

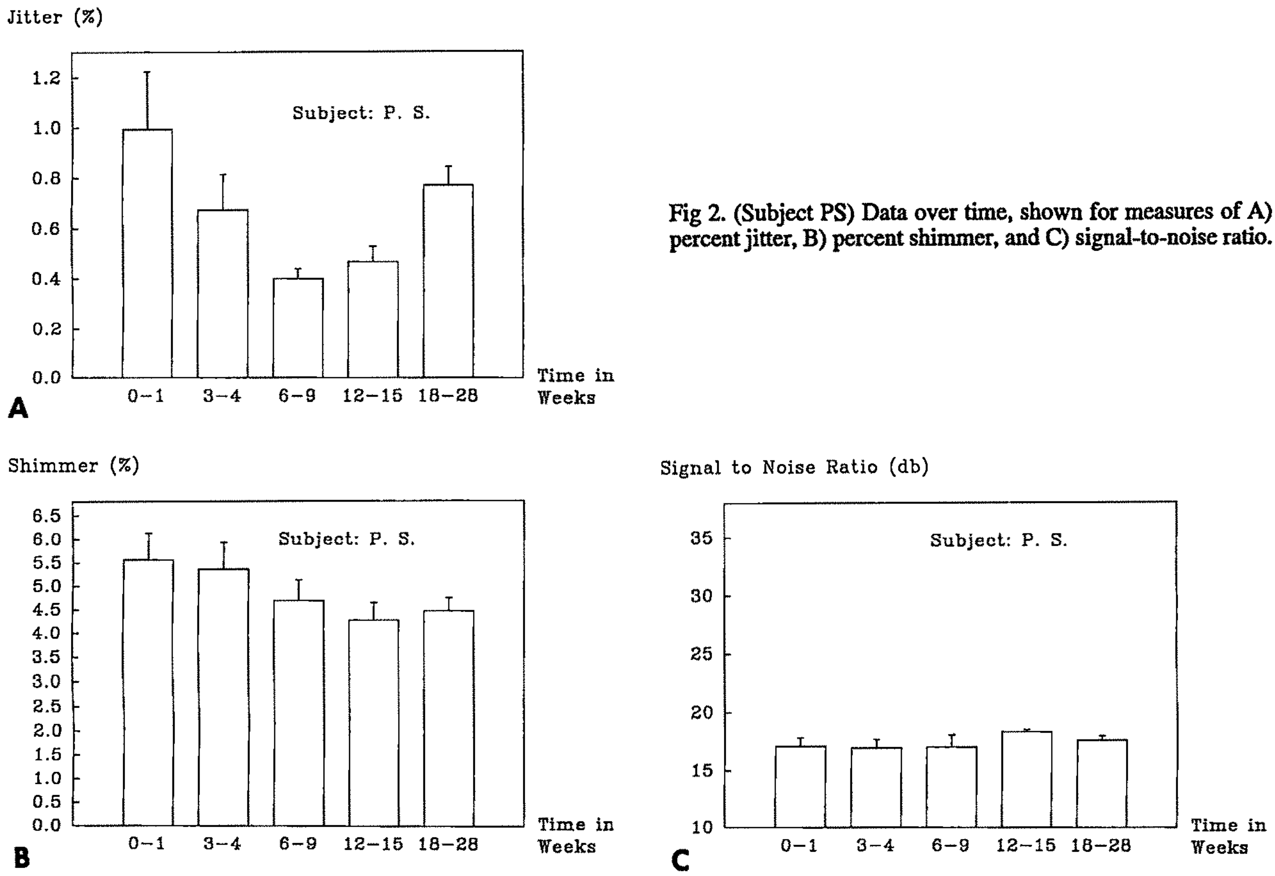


Fig 2. (Subject PS) Data over time, shown for measures of A) percent jitter, B) percent shimmer, and C) signal-to-noise ratio.

of the listeners' scores was computed and used as the perceptual score for a given voice sample.

After rating each sample on overall acceptability, the listeners repeated the task by rating each voice sample on degree of strain, breathiness, and roughness, with a score of 0 indicating none of the designated voice quality and 100 indicating a voice characterized completely by the specific voice quality. Repeated samples were used to ensure an acceptable level of repeatability for the measures. Interjudge agreement on measures for the same sample was >80%.

For statistical analysis, nonparametric procedures were used, since the visual analog scale produces ordinal rather than interval data. The Friedman analysis of variance test was used to determine group changes in voice quality over time; the Wilcoxon paired comparisons test was used to assess voice quality differences between specific evaluation points.

The acoustic signal from each voice recording was played back and digitized on a National Instruments AT-MIO-16F board at 12-bit resolution with a 20-kHz rate. The acoustic analysis was also blind to clinical treatment information. Each digitized file was viewed, and a representative 2-second sample of phonation was selected for each analysis. Segments were analyzed for three separate voice samples from each recording session, and the perturbation measures from these samples were averaged. Digitized samples were analyzed for percent of jitter, percent of shimmer, and signal-to-noise ratio by means of Cspeech software. Because there was large variation in study entry sample values of jitter, shimmer, and signal-to-noise ratio among the subjects, the entry visit voice recording data were used as a baseline to which subsequent-visit data were compared for each patient. Analysis of variance and paired *t*-tests were used to detect change over time.

RESULTS

Individual data are shown for a typical patient in Figs 2 and 3. This patient (subject PS) was a 42-year-old professional who presented complaining of chronic sore throat, recurring hoarseness, and a sensation of postnasal drip. He had moderate symptoms of heartburn during the day and evening and occasionally awoke at night with coughing. His throat soreness was worse on awakening in the morning and his hoarseness progressed during the day, deteriorating with voice use. A 90° magnifying telescopic examination of his larynx revealed notable erythema and hypervascularity of the mucous membranes over the

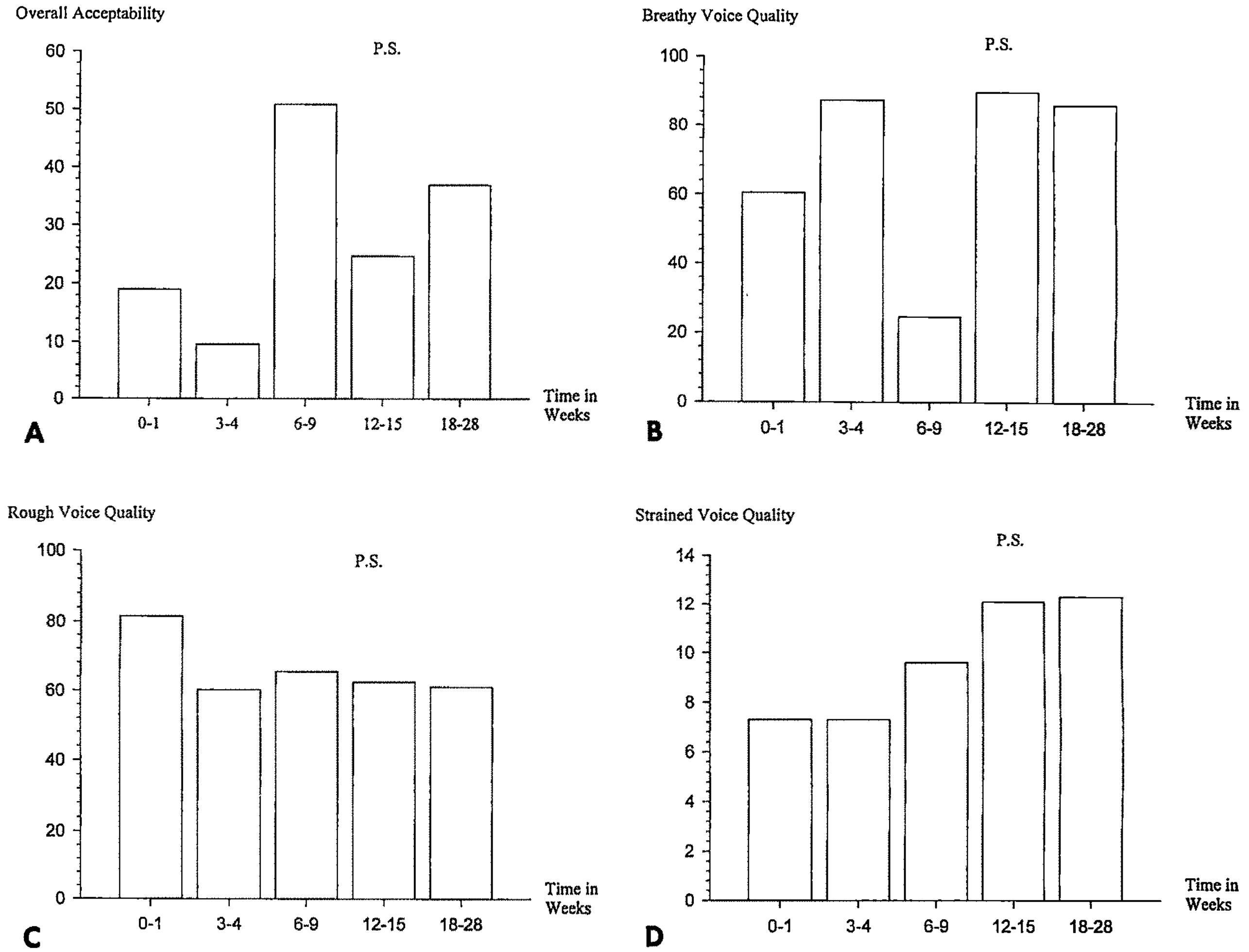


Fig 3. (Subject PS) Data over time, shown for perceptual judgments of trained listeners rating acceptability of A) voice quality, B) breathiness, C) roughness, and D) strain.

posterior laryngeal structures, with a mucosal burn appearance over the posterior glottic wall. The epithelium over the vocal processes was inflamed. There was moderate edema and some hypervascularity of the vocal folds. The patient was treated with omeprazole 20 mg before retiring and nocturnal antireflux precautions, avoiding food or liquid ingestion for 2 hours before retiring and elevating the head and shoulders during sleep. He was also advised to avoid clearing his throat and was counseled about voice conservation precautions. With treatment, his symptom reduction indicated response to treatment at 3 weeks, with resolution of complaints at 6 weeks. At 6 weeks, 90° telescopic laryngoscopy showed resolution of the erythema and mucosal burn appearance. The vocal folds appeared essentially normal, although some hypervascularity of the supraglottic mucosa persisted. At 6 weeks, he stopped taking omeprazole, while continuing nocturnal antireflux precautions. At the 12-week examination, he continued to be asymptomatic. At the 18-week examination, he complained of recurrence of sore throat and recurrence of some intermittent hoarseness.

Acoustic analysis of voice recordings showed a progressive reduction in measure of percent shimmer to 6 weeks during antisecretory treatment. Twelve weeks after stopping acid suppression medication, there was a slight increase in percent shimmer. Percent jitter showed a greater sensitivity to treatment, decreasing up to the time of the 6-week examination, at which time gastric acid suppression was discontinued. Thereafter, at 12- and 18-week examinations there was an increase again in the measured percent jitter. The signal-to-noise ratio improved slightly at 12 weeks and deteriorated at 18 weeks, with return of symptoms after cessation of acid suppression. The perceptual data (Fig 3) showed that acceptability was most closely correlated with percent jitter. Subject PS demonstrated low levels of overall acceptability at evaluations 1 and 2, and at the third evaluation, overall acceptability increased markedly, to a moderately high level. At the fourth and fifth evaluations, overall voice acceptability dropped to moderately

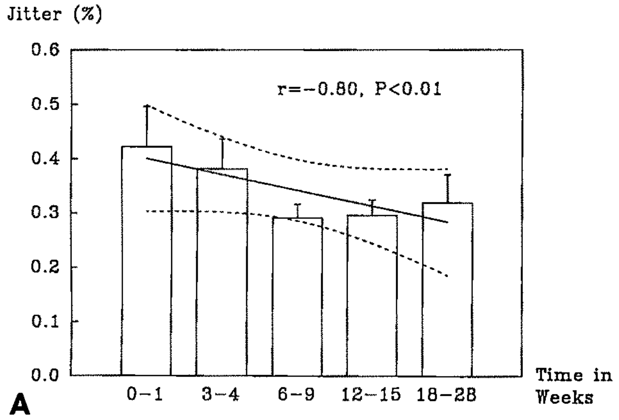
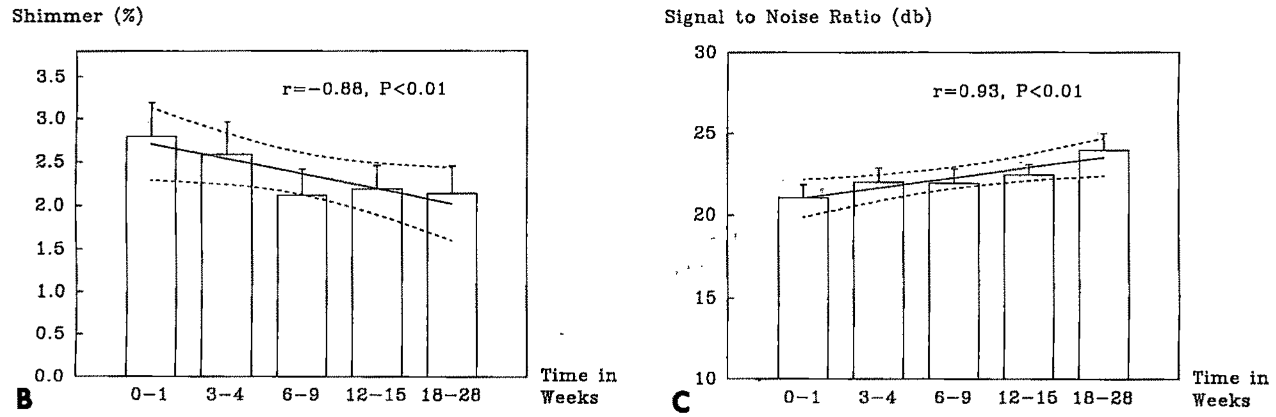


Fig 4. Group data for acoustic measures. Overall change was significant at p < .01 level, even though there was some relapse of laryngitis in some patients after acid suppression was stopped at 3 to 6 weeks. A) Jitter. B) Shimmer. C) Signal-to-noise ratio.



low levels. This subject's voice was also characterized by a steady increase over time in a strained voice quality and a general reduction in rough voice quality. The perceptual data therefore showed improvement with treatment and overall rebound of abnormality after cessation of treatment.

Although there was often generally good agreement of perceptual data with acoustic perturbation measures for individual patients, there were no significant group differences across evaluation points for any of the perceptual voice quality assessments. Also, statistical analysis did not show a high correlation of the measured acoustic perturbations with either perceptual assessments of overall voice abnormality or perceptual assessments of a particular voice quality. The large standard deviations and small number of subjects contributed to the lack of significance.

The patients in the study varied in degree of symptoms and apparent laryngitis; therefore, the large standard deviations are expected. Two patients who complained of hoarseness had very mild evidence of laryngitis and minimal hoarseness. The data for these subjects showed a prompt response to treatment, with reduced jitter and shimmer and increased signal-to-noise ratio at 3 weeks. At the other extreme, a patient with severe laryngitis and notable hoarseness did not respond to the levels of omeprazole used in this study and had persistent symptoms and hoarseness throughout the duration of the study.

The perceptual data were highly individualized, as there was a wide range of voice acceptability at the start of treatment. Group data were not significant, probably because of high standard deviations and relatively small numbers of subjects. Comparing the perceptual assessment data among the different tasks showed that the rating of overall voice acceptability was most highly correlated with the assessment of rough voice quality ($r = -.66$); as perception of roughness increased, the acceptability of the voice decreased.

The longitudinal group data for the acoustic measurements of percent jitter, percent shimmer, and signal-to-noise ratio for all 6 patients are shown in Fig 4. Initial percent jitter, percent shimmer, and signal-to-noise levels varied among the patients, as was expected. Longitudinal data, however, demonstrated a clear trend of change in the objective measures with treatment. The group data show that percent jitter, percent shimmer, and signal-to-noise ratio changed significantly toward less perturbation of the acoustic signal during antisecretory treatment. Percent jitter and shimmer correlated negatively with time ($r = -.8$, $p < .01$), ie, decreased during treatment, and signal-to-noise ratio correlated positively with time ($r = .93$, $p < .01$). Most patients had experienced resolution of symptoms by 6 to 9 weeks and discontinued omeprazole treatment at that time. The data show some deterioration in voice perturbation measures after 6 to 9 weeks. This correlated with a recurrence of laryngitis symptoms. However, the overall significant improvement in the objective measures held, even with some deterioration in measures after antacid treatment ceased.

DISCUSSION

Cherry and Margulies[7] first suggested that gastric acid plays a role in causing contact ulcers and chronic laryngeal irritation. Subsequently, a number of reports have addressed the possible role of refluxed gastric acid in causing laryngeal disease.[8-18] Experience with a larger prospective outcome study of the efficacy of antireflux precautions and gastric acid suppression for the treatment of symptoms and signs of chronic laryngitis indicates that the chronic laryngitis symptoms of most patients respond to antireflux and antacid treatment.[1] Complaints of dysphonia vary in the population of patients with chronic posterior laryngitis. In many cases, although the patients complain of recurring hoarseness, their voices may not be notably abnormal to the clinician. The acoustic and perceptual measures obtained here demonstrated this wide range of variation. The mean perturbation values were somewhat higher than might be expected for a group of normal voices, and this abnormality was detected by the trained listeners, even though the perturbation values in general were within a range that might include normal asymptomatic speakers.[19] Particularly when hoarseness symptoms are relatively subtle, changes in voice quality over time may be difficult to assess, even when the clinician is an experienced listener. However, acoustic perturbation measures for a given speaker appear to be reproducible over time and are likely to be a reasonable way of following change in voice perturbation.

This study demonstrated that objective measures of voice perturbation, such as acoustic measures of jitter, shimmer, and signal-to-noise ratio, document change in vocal quality with treatment for chronic laryngitis. These measures also show evidence of some deterioration in vocal quality when acid suppression treatment is stopped. Although they are important clinical assessments, patient symptoms and laryngeal visual examination findings are relatively subjective measures of treatment outcome. Acoustic perturbation measures made from voice recordings provide objective measurement of voice changes that may not be evident on the usual subjective clinical evaluations. Objective measures of voice perturbation demonstrate a clear treatment response to antireflux and antisecretory treatment for hoarseness associated with chronic posterior laryngitis.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria (in English July 3-5, 1997, and in German September 4-6, 1997). For more information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax 43/316/385/3425.

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PHAGOPHOBIA: A FORM OF PSYCHOGENIC DYSPHAGIA

A NEW ENTITY

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There is a group of patients presenting with either acute or chronic dysphagia secondary to fear of swallowing. We have termed this entity phagophobia. It is characterized by various significant swallowing complaints in the face of normal findings on head and neck examination, oropharyngeal swallowing videofluoroscopy, and standard barium swallow study. Ten patients who received diagnoses of phagophobia after a full evaluation at our swallowing disorders center are presented. Each patient was then evaluated by a psychologist, and an attempt at therapy was undertaken. We discuss the specific clinical features, assessment, and treatment of this frequently misdiagnosed disorder.

KEY WORDS — choking phobia, deglutition disorders, psychogenic dysphagia, swallowing disorders.

Phagophobia, a fear of swallowing, is a form of psychogenic dysphagia. It is characterized by various significant swallowing complaints with normal physical examination and laboratory findings. We will discuss the clinical features, as well as strategies for the assessment and treatment, of this disorder.

Patients with dysphagia, as defined by difficulty swallowing regardless of cause, often initially present to the internist, gastroenterologist, or otolaryngologist. Phagophobia is defined by patients as the feeling that the bolus is stuck in the throat; however, when questioned, patients acknowledge that the bolus does not actually stick. They therefore do not require maneuvers to move the bolus. Organic (nonpsychogenic) dysphagia is secondary to problems with either bolus propulsion or aspiration. In the case of abnormal bolus propulsion, patients describe the bolus as getting stuck; bolus passage either then occurs spontaneously after a varying time interval or requires maneuvers such as multiple liquid or dry swallows or even pharyngeal regurgitation to move the bolus retrograde into the oral cavity. Aspiration, on the other hand, can cause coughing and the sensation of the bolus "going down the wrong passageway." Sometimes the aspiration is silent and is recognized only after it results in aspiration pneumonia.

Many authors have described patients with choking phobia.[1] It is a confusing term, because patients often do not distinguish difficulties with bolus propulsion versus aspiration; they may refer to either problem as choking, whereas clinicians define choking as a symptom of aspiration. For this reason it is crucial to ask the patient detailed questions regarding dysphagia symptoms; the term choking should probably not be used by clinicians.

Other associated complaints of phagophobic patients include a feeling of throat pressure, constriction, or closing, and difficulty initiating the swallow. Some, but by no means all, patients with phagophobia also describe a foreign body sensation in the throat (globus pharyngeus) that is usually present at rest and is often unchanged or even ameliorated by a bolus swallow. Globus is distinguished from phagophobia by its perception of noninterference with the actual swallow.

Many patients report weight loss secondary to decreased oral intake. However, unlike patients with the eating disorders anorexia nervosa and bulimia nervosa,[2] phagophobics do not intentionally lose weight and are displeased with their changed body weight. Similarly, phagophobics do not have a distorted body image; they perceive their shape and size accurately.

There have been several case reports and discussions about the role of psychiatric problems in aspects of swallowing dysfunction such as esophageal dysmotility.[3,4] This paper describes patients with no organic disorder who have significant dysphagia.

MATERIALS AND METHODS

Between January 1993 and September 1996, 257

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TABLE 1. HISTORY FOR DYSPHAGIA PATIENTS

Duration of symptoms
Sensation or occurrence of bolus sticking
Localization of where bolus feels stuck
Maneuvers required to move bolus
Sensation of aspiration; coughing
Weight loss or dietary modification
Symptoms of eating disorders
Relevant antecedent events or current stressors
Family history of dysphagia
Related symptoms
  Globus, odynophagia, nasal regurgitation, ptosis, diplopia, dysarthria, dysphonia, diffuse muscle weakness, heartburn

patients with dysphagia were seen by the first author. The patients were all referred by primary care physicians or other specialists, including gastroenterologists, neurologists, general or thoracic surgeons, or neurosurgeons. Many were referred by other otolaryngologists.

Patients were evaluated with history-taking, physical examination, and laboratory tests. A detailed dysphagia history was obtained from each patient (Table 1), including the sensation or actual occurrence of the bolus sticking, and if so, what bolus consistency and what maneuvers were required to remove the bolus. The patient was asked to try to localize and point to where the bolus felt stuck. Inquiry was made as to whether the patient experienced any sensation of the bolus entering the airway, including coughing, for example. The presence of weight loss or dietary modification was ascertained, along with symptoms of anorexia nervosa or bulimia nervosa. In addition, the patient was asked about any relevant antecedent events such as actual aspiration, either experienced or witnessed by the patient, as well as stressful life events. Any family history of dysphagia was also obtained. Query for other pertinent symptoms included odynophagia, nasal regurgitation, ptosis, diplopia, dysarthria, dysphonia, diffuse muscle weakness, and heartburn.

Physical examination included a thorough assessment of the oral cavity, including tongue strength and mobility and palatal elevation. The nasopharynx, oropharynx, and hypopharynx were visualized with both a mirror and a flexible fiber-optic laryngoscope. In addition to looking for any structural lesions, particular attention was paid to vocal cord mobility and the presence of any pooled secretions in the pharyngeal recesses. The neck was then palpated.

All patients complaining of dysphagia underwent an oropharyngeal videofluoroscopic swallowing evaluation. Normal findings would rule out any significant oropharyngeal swallowing dysfunction. If these findings were normal, a standard barium swallow study with both barium liquid and pill followed at the same sitting to reveal distal disorders such as esophageal webs, strictures, or rings and esophageal dysmotility. One could argue that further evaluation such as esophagogastroduodenoscopy or esophageal motility studies should be performed; however, with no abnormalities on the detailed history, physical examination, or swallowing studies outlined above, further testing is unnecessary.

A diagnosis of phagophobia was made when normal head and neck examination findings along with normal findings on videofluoroscopy and barium swallow study were made. Regarding the history, any patients with actual rather than perceived difficulty moving the bolus were excluded; ie, there could be no history of requiring regurgitation or other maneuvers to move the bolus. Also, anyone with odynophagia, spontaneous coughing during the swallow, nasal regurgitation, diplopia, dysarthria, paresis, uncontrolled heartburn, anorexia nervosa, or bulimia nervosa was excluded. If, after this thorough evaluation, no organic disorder was found, patients were offered an evaluation with the second author for the treatment of phagophobia using nonpharmacologic methods. Only a small percentage of patients chose this option. Reasons for not following through varied among patients and included financial concerns, aversion to psychologic rather than medical treatment, and logistic (travel) concerns. In a few cases, patients stated that they wanted to see one more medical specialist "just to make sure" that nothing was missed in their previous workups.

The evaluation by the psychologist included a detailed psychiatric history, as well as an assessment of current symptoms, to ascertain the presence of a psychiatric diagnosis. In addition, detailed questions concerning the onset of the disorder, precipitating and maintaining factors, and consequences to the patient's physical and psychological well-being were asked in order to better understand the role that phagophobia played in the patient's life. Questions regarding the patient's beliefs about the cause of the disorder were asked in an effort to understand the patient's willingness and motivation to participate in nonmedical treatment. Patients were given the opportunity to discuss any feelings about the lack of medical findings and were encouraged to give voice to concerns that medical professionals had not yet been able to find a medical cause because they had not used the "proper tests." The evaluation was concluded by explaining to the patient the rationale for using nonpharmacologic methods, ie, that such treatment may help the patient cope with the symptoms by using the mind to treat the body, and that techniques such as relaxation can reduce muscle tension that

TABLE 2. CLINICAL CHARACTERISTICS OF TEN PATIENTS WITH PHAGOPHOBIA

Pt No.	*Age*	*Duration*	*Presenting Complaint*	*Event*	*Weight Loss*	*Diet Change*
1	41	1 mo	Difficulty breathing, not able to swallow, lump in throat	Sick parent	Unknown	None
2	36	2 y	Trouble swallowing, food sticks in throat, pressure in throat	Stressful work	Unknown	Liquids and soft foods
3	19	1 y	Food stuck in throat, throat closes up and tightens, fear of choking	Choked on chicken	4.5 kg over 3 mo	Liquids and soft foods
4	26	2 y	Food stuck in throat, fear of choking	None	20.5 kg	Liquids only
5	22	3 y	Food stuck in throat, afraid can't breathe, fear of choking	Saw child choke	11.4 kg in 1 mo	No dairy products
6	25	6 y	Restriction in throat, difficulty swallowing	None	Unknown	None
7	36	4 y	Food gets stuck in throat, can't swallow anything solid	Choked on chicken	Unknown	Liquids only
8	37	6 mo	Food gets stuck in throat, can't eat any solid food	Couldn't swallow mint	22.7 kg over 4 mo	Liquids only
9	32	1 mo	Trouble swallowing solid food, afraid will choke	Bread stuck in throat	2.3 kg	Liquids and soft foods
10	34	4 y	Difficulty swallowing, food stuck in throat, difficulty initiating swallow	Candy stuck in throat	5.9 kg	No meat

may be related to the difficulties with swallowing. It is important to assess the patient's response to this rationale and any resistance to a treatment in which he or she plays an active role.

RESULTS

We present data from 10 patients with phagophobia (Table 2), which included 7 women and 3 men, ranging in age from 19 to 41. The duration of symptoms prior to presentation to the physician ranged from acute (1 month) to chronic (4 to 6 years). Each patient presented with difficulty swallowing, described as a feeling of the bolus sticking in the throat. None of the patients actually had an inability to move the bolus. Four of the 10 patients described a fear of choking as defined by aspiration-related symptoms; in each of these cases, there had been an episode or a witnessing of choking some time in the past. None of the patients had ongoing symptoms of aspiration such as coughing. Only 1 patient had globus. One patient had a family history of dysphagia. There was no history of odynophagia, nasal regurgitation, ptosis, diplopia, dysarthria, dysphonia, diffuse muscle weakness, heartburn, anorexia nervosa, or bulimia nervosa in any of the patients. Six patients had lost weight secondary to their swallowing difficulties. In addition, dietary changes to accommodate the perceived dysphagia were very common (8 patients), and 3 patients consumed only a liquid diet. The head and neck examination findings were entirely normal in all patients. In addition, all results of laboratory studies were normal, including videofluoroscopy and barium swallow study. None of the patients met formal diagnostic criteria for any psychiatric disorder, though all had mild depression and anxiety. Although psychologic treatment was recommended for all 10 patients, only 3 followed through beyond the initial evaluation. For these 3 patients, the outcome after treatment was successful in that the symptoms remitted in each case. Follow-up of these 3 patients 6 to 12 months later revealed that they remained largely symptom-free with occasional brief relapses during times of stress.

Because the initial history, physical examination, videofluoroscopy, and barium swallow study findings were all normal, these tests were not repeated. All patients were asked to return if they noted any increase or change in their symptoms.

CASE REPORT

We will discuss one case (patient 3) of successful treatment of phagophobia and describe the behavioral techniques and the use of hypnosis that proved helpful in symptom relief. The patient was a 19-year-old college student who reported a 1-year history of phagophobia. Her symptoms, which began whenever she started to eat, included a sensation of her throat tightening, difficulty breathing, a feeling that food became stuck in her throat, and a fear of the food going into her airway. She had never actually aspirated or been unable to pass the bolus. She ate only soft foods and liquids; she had lost 4.5 kg over a 3-

month period prior to presentation to the otolaryngologist (J.S.). The patient had a psychiatric history significant for depressive symptoms in the past for which she had been treated with antidepressant medication. In addition, she described one panic attack unrelated to swallowing for which Xanax was prescribed. Her head and neck physical examination, swallowing videofluoroscopy, and barium swallow study findings were normal. She presented to the psychologist (D.L.F.) as a slightly depressed young woman who seemed eager to obtain some symptom relief and appeared motivated to play an active role in treatment. She seemed accepting of her various physicians' reports that no medical cause for her disorder could be discerned, and she was open to behavioral work and hypnosis to address her symptoms.

Psychological treatment consisted of four aspects: relaxation training, cognitive therapy, hypnosis, and homework assignments that included self-monitoring of food intake, anxiety level, and thoughts. It began with progressive muscle relaxation, in which the patient tensed and relaxed each muscle group. She was also taught diaphragmatic breathing in conjunction with relaxation. Cognitive therapy involved an examination of thoughts that occurred before and after eating and a suggestion to counter negative thinking with more positive self-statements. Thoughts such as "I'm waiting for the choking to happen" and "I just know once I start to eat the food is going to get stuck" were challenged and replaced with statements such as "If I relax while I'm eating, the food will go down smoothly, my breathing will be fine, and I will not choke."

Hypnosis combined both relaxation and cognitive therapy by offering appropriate suggestions to the patient while in a relaxed state. Suggestions for specific neck, shoulder, and throat muscle relaxation were made, along with reminders that nothing was actually stuck in her throat. The patient was asked to visualize the muscles in her throat "opening up" to allow the food to pass. Audiotapes were made during the hypnosis sessions and the patient was encouraged to listen to them each day before her midday and evening meals.

After four visits utilizing these techniques, the patient reported considerable progress. She had listened to the tapes daily and recorded her food intake, thoughts, and degree of anxiety experienced. She noticed changes in several areas, including a significant reduction in her fear of eating and choking, a return to eating some solid foods, and an ability to relax during a meal and to continue eating if she became tense. This progress continued over the next several months. The patient recontacted the psychologist 4 months later with a return of symptoms secondary to going back to college and feeling unhappy about this. She returned for two visits, during which similar interventions were employed, and her symptoms remitted again. The patient has returned on two occasions, spaced by 1 year, for several of these "booster" sessions, which proved to be helpful in alleviating the return of the symptoms of phagophobia.

DISCUSSION

We believe that phagophobia is a discrete entity and should be placed in the differential diagnosis of any patient presenting with dysphagia. The patients present to the physician with a sensation of being unable to pass the bolus, sometimes accompanied by a fear of aspiration. Although others have defined this patient group as having a fear of choking, in our experience this is too narrow a definition because it does not encompass all of the patients with swallowing fears. There is sometimes a precipitant to the swallowing difficulties, such a witnessed choking incident, but this is not always the case. When there has been a witnessed event, this is more likely to lead to a fear of aspiration in addition to a fear of not being able to move the bolus. Because these patients tend to somaticize and believe there is true organic disease, they rarely present to a mental health professional.

Phagophobia, although a distinct diagnostic entity, is a diagnosis of exclusion. Other authors[3] have noted a high incidence of underlying organic disease in dysphagia patients who did not initially undergo a thorough evaluation. It is imperative that the clinician perform a detailed history (Table 1), physical examination, swallowing videofluoroscopy, and barium swallow study to rule out organic disease. This is especially important in view of the phagophobic patient's belief in the organic nature of the problem.

As is the case with many disorders that present with physical symptoms yet lack an organic cause, treatment is often difficult at best. We have noticed that phagophobic patients are extremely reluctant to give up their search for a physical explanation, even in the face of repeated negative laboratory findings. They often see many different specialists (otolaryngologists, gastroenterologists, allergists) in an effort to find the doctor who will discover the medical reason for their difficulties. It is important to some of these patients to be seen by medical and mental health professionals who have significant expertise in the evaluation and treatment of swallowing problems.

After organic causes have been ruled out by history, physical examination, swallowing videofluoroscopy, and barium swallow study, patients are referred to a psychologist for treatment. The manner in which this referral is made is very important. Because these patients are highly invested in finding a physical cause, the referral must be made in such a way that patients are not left feeling as though the physician believes their problems are "all in their head." Instead, the physician should explain that there are some techniques available to help the patient learn to cope with the symptoms, and since no medical explanation of the cause has been found, such techniques may be successful in alleviating their symptoms. Reassurance that there is no neoplastic, structural, or neurologic disorder is helpful to some patients. Even when the diagnosis is tactfully stated, however, it is our experience that only a minority of patients will follow through with the mental health referral.

There have been many reports in the literature of psychiatric patients who also have dysphagia.[4-6] Patients with phagophobia may have underlying psychiatric symptoms, primarily related to anxiety and depression, but generally do not have a formal psychiatric diagnosis.[7,8] They often do have difficulties managing the stress in their lives, and it may be that their phagophobic symptoms can be understood, in part, as an effort to channel their anxiety into their bodies.

Because this entity has not been formally defined or fully recognized by both medical and mental health professionals, little is known about the treatment. There are some anecdotal reports of various techniques such as biofeedback used in the treatment of psychogenic dysphagia.[9] As we gain more experience with this group of patients, additional techniques such as progressive muscle relaxation and hypnosis will need to be evaluated and their outcomes studied objectively.

In summary, there is a group of patients whose primary problem is fear of swallowing. For this reason, we have named this entity phagophobia. It is an important diagnosis to make in order to focus the medical and psychiatric communities on diagnostic and treatment strategies that can benefit patients with this disorder.

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FUNCTIONAL OUTCOME AND PROGNOSIS FACTORS AFTER SUPRACRICOID PARTIAL LARYNGECTOMY WITH CRICOHYOIDOPEXY

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A retrospective analysis of the medical records and operative files of 124 patients who consecutively underwent supracricoid partial laryngectomy with cricohyoidopexy (SCPL-CHP) was undertaken. The objective of this study was to analyze the postoperative course and functional results of SCPL-CHP. The mean duration of follow-up was 7 years (range 1 to 20 years). The postoperative mortality was 1.6%. The average times until tracheostomy and nasogastric feeding tube removal were 8 and 22 days, respectively. All patients but 1 were decannulated. The rate of pneumonia from aspiration was 11.5%. Completion total laryngectomy and permanent gastrostomy were required in 2.5% and 2.5% of patients, respectively. Overall normal swallowing without permanent gastrostomy and respiration without tracheostomy were achieved by the first postoperative year in 91% and 99.1% of patients, respectively. Univariate analysis of the potential correlation between various variables and the duration of the tracheostomy and nasogastric feeding tube, the incidence and causes of mortality, the incidence and type of the various complications, the recovery of swallowing, and the length of hospitalization is presented. The report also discusses the management and follow-up care.

KEY WORDS — cricohyoidopexy, endolaryngeal carcinoma, supracricoid partial laryngectomy.

INTRODUCTION

Since the 1970s, supracricoid partial laryngectomy with cricohyoidopexy (SCPL-CHP) has been performed in our department for selected transglottic and supraglottic squamous cell carcinoma.[1-4] The procedure is presented as an alternative to radiotherapy (RT), horizontal supraglottic laryngectomy (SGL), and near-total and total laryngectomy in selected cases of laryngeal carcinoma.[1-3] The oncologic value and limitations of SCPL-CHP have been reported by various authors.[1-13] However, no report based on a large number of patients with a long-term follow-up has analyzed the functional outcome and complications after SCPL-CHP.

The present retrospective study was designed to analyze the postoperative course, mortality, morbidity, and short- and long-term functional results after SCPL-CHP. Speech and voice analysis will not be discussed in the present report. Univariate analysis of the potential correlation between a number of variables and the duration of use of the tracheostomy and nasogastric feeding tube, the incidence and causes of mortality, the incidence and type of the various complications, the recovery of swallowing, and the length of hospitalization is presented. This article furthermore discusses the management and follow-up care after SCPL-CHP completion.

MATERIALS AND METHODS

One hundred twenty-four patients underwent SCPL-CHP at our department during the period from March 1974 to April 1993. One hundred eighteen men and 6 women were included. Their ages ranged from 33 to 73 years, with an average of 55 years. Their past medical history is presented in Table 1. All patients were smokers, with a mean of 42 pack-years (range 5 to 176). Only 16.9% (21/124) of patients did not consume alcohol. The TNM classification according to the 1988 American Joint Committee on Cancer staging system[14] is summarized in Table 2.

The SCPL-CHP technique will not be described in detail, since it has been previously reported.[4]

In this series, SCPL-CHP was associated with radical or conservative neck dissection in 97.6% (121/124) of patients. Resection of one arytenoid cartilage was performed in 50.8% (63/124) of patients. Pyriform sinus repositioning was performed in 12.9% (16/124) of patients. A cricopharyngeal my-

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TABLE 1. PAST MEDICAL HISTORY (124 PATIENTS)

	Patients	
Past Medical History	*No.*	*%*
Gastroesophageal reflux	22	17.7
COPD	21	16.9
Duodenal ulcer	20	16.1
Arteritis	19	15.3
High blood pressure	16	12.9
Coronary artery disease	9	7.3
Depression	9	7.3
Laënnec's cirrhosis	6	4.8
Diabetes mellitus	6	4.8
Pulmonary tuberculosis	4	3.2
Other	6	4.8

COPD — chronic obstructive pulmonary disease.

otomy was associated in 3.2% (4/124) of patients. Prophylactic antibiotics were delivered in all patients. Preoperative laryngeal radiotherapy to the larynx was performed in 2.4% (3/124) of patients. Postoperative RT was performed in 33.8% (42/124) of patients. The postoperative dosages delivered to the larynx and lymph nodes varied from 35 to 60 Gy and 35 to 67 Gy, respectively.

Postoperatively, the tracheostomy tube was removed as soon as possible. The first attempt was on the third postoperative day. The nasogastric feeding tube was removed once the tracheostomy site was healed in patients able to swallow their own saliva. The patient was then encouraged to start eating transorally on a supraglottic diet. Speech therapy was initiated by the end of the first postoperative week. Daily chest therapy was performed during the first postoperative month.

Evaluation of swallowing disorders included a careful observation of the patient by the physician, with or without a videolaryngoscopy. Grading of postoperative aspiration was performed according to the scale used by Leipzig[15] and Pearson[16] (0 = no, 1 = occasional cough but no clinical problem, 2 = constant cough worsening with meals or swallow, 3 = pulmonary complications).

Mortality was considered as related to surgery if related to a postoperative complication or if occurring during the first postoperative month in patients without complications.

TABLE 2. TNM STAGING
(124 PATIENTS INITIALLY M0)

	N0	*N1*	*N2*	*N3*
T1	2	0	0	0
T2	41	7	4	1
T3	47	12	4	1
T4	3	1	1	0

The minimum follow-up in our series was 16 months, with a mean of 7 years. The following variables — age, sex, general status, preoperative radiotherapy, duration of surgery, arytenoid cartilage resection, neck dissection, cricopharyngeal myotomy, pyriform sinus repositioning, and postoperative RT — were tested for potential correlation with the duration of tracheostomy and nasogastric feeding tube, swallowing rehabilitation, postoperative complications, cause of death, and length of hospitalization. Patients who died in the postoperative period were excluded from this analysis. Calculations were done with PCSM (Programme Conversationnel pour les Sciences et le Marketing, Deltasoft, Inc, Meylan, France) statistical software and the χ^2 test, the nonparametric Mann-Whitney U test and Kruskal-Wallis H test, and the linear regression with Pearson correlation.

RESULTS

Mortality and Morbidity (124 Patients). Two patients died postoperatively. The cause of death was a ruptured abdominal aortic aneurysm in 1 patient and pneumonia from aspiration associated with distant bone metastasis in the other patient, in whom SCPL-CHP was performed as a salvage procedure after failed laryngeal RT. There was no significant statistical relationship between occurrence of death and the variables under analysis.

Medical complications were noted in 11.3% (14/124) of patients (Table 3). None of the variables analyzed was statistically related to the incidence of medical complications.

Surgical complications are presented in Table 3.

TABLE 3. POSTOPERATIVE COMPLICATIONS
(122 PATIENTS)

	Patients	
Postoperative Complications	*No.*	*%*
Surgical complications		
Wound infection	10	8.2
Cervical bleeding	4	3.3
Hematoma	2	1.6
Laryngeal necrosis	5	4.1
Laryngeal stenosis	2	1.6
Retrocricoid ulceration from nasogastric feeding tube	2	1.6
Medical complications		
Digestive hemorrhage	4	3.3
Severe COPD	3	2.5
Diabetes mellitus	3	2.5
Cardiac complications	3	2.5
Ulcer perforation	1	0.8

COPD — chronic obstructive pulmonary disease.

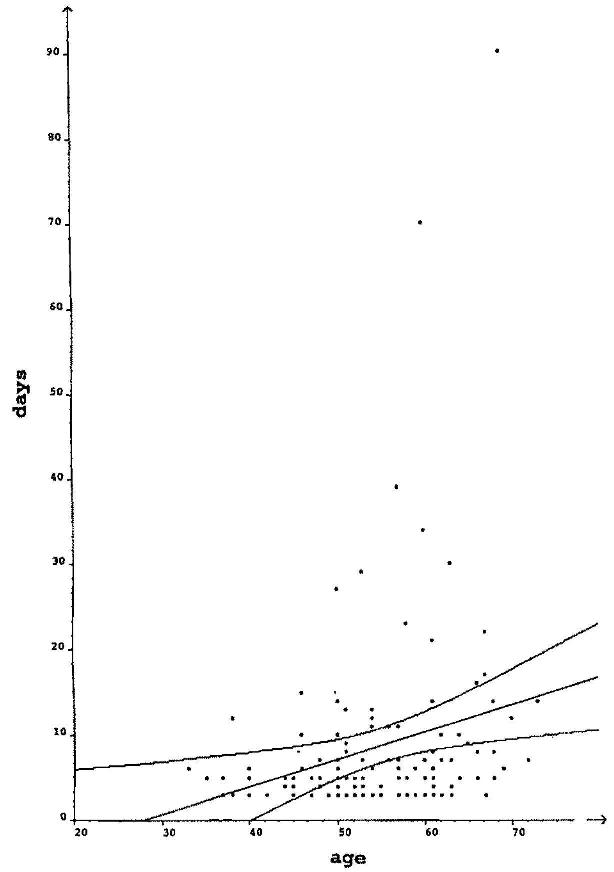

Fig 1. Duration of tracheostomy tube versus age (122 patients, Pearson's correlation).

Pharyngeal fistula and ruptured pexy were not encountered. Laryngeal necrosis resulted in delayed tracheostomy tube removal and required a transient percutaneous endoscopic gastrostomy in 2 patients. Successful management of laryngeal stenosis without the need for a permanent tracheostomy was achieved in 1 of these patients. A permanent tracheostomy was required in the other patient.

Neck dissection was the only variable that statistically increased the risk for wound infection (p = .02), cervical bleeding (p = .001), and hematoma (p = .001).

Respiration (122 Patients). All patients but 1 were decannulated. This patient, with severe chronic obstructive pulmonary disease (COPD), had the tracheostomy tube maintained for 60 days and ultimately developed laryngeal stenosis.

In the remaining 121 patients, the average time until decannulation was 8 days (range 3 to 90 days). The delay for decannulation was statistically more likely to increase with age (Fig 1, p = .007), postoperative laryngeal edema (p = .001), and preoperative COPD (p = .006). The extent of removal of the arytenoid cartilage did not demonstrate a significant relationship to the duration of the tracheostomy tube (Table 4). There was no significant difference in the time of decannulation between the irradiated and nonirradiated patients (Table 4).

TABLE 4. UNIVARIATE ANALYSIS OF RELATIONSHIP BETWEEN DELAYED DECANNULATION, ASPIRATION, AND VARIABLES UNDER ANALYSIS (122 PATIENTS)

		Aspiration	
Variables	*Delayed Decannulation*	*Grade 1 or 2*	*Grade 3*
Age	.004	.07	.1
Sex	.16	.3	.3
Preoperative COPD	.006	.07	.09
Diabetes mellitus	.2	.09	.08
Arteritis	.4	.3	.2
Gastroesophageal reflux	.5	.11	.13
Preoperative RT	.3	.08	.09
Arytenoid cartilage resection	.2	.07	.06
Neck dissection	.8	.4	.2
Cricopharyngeal myotomy	.12	.1	.13
Pyriform sinus repositioning	.2	.05	.07
Transection of superior laryngeal nerve	.5	.09	.07
Postoperative RT	.1	.09	.11

Data are p values.

COPD—chronic obstructive pulmonary disease, RT—radiotherapy.

Postoperative laryngeal edema was noted in 14.8% (18/122) of patients. Successful management required oral steroids and carbon dioxide laser vaporization in 8 and 4 patients, respectively.

Deglutition (122 Patients). The nasogastric feeding tube was left in place, on average, for 22 days (range 13 to 60 days). The duration of the nasogastric feeding tube was statistically more likely to increase with COPD (p = .02), diabetes mellitus (p = .04), and increased delay for tracheostomy tube removal (p = .003).

Successful deglutition by the first postoperative month was achieved in 45.1% (55/122) of patients.

Temporary grade 1 or 2 aspiration occurred in 43.4% (53/122) of patients. None of the variables under analysis was statistically related to grade 1 or 2 aspiration incidence. Grade 1 or 2 aspiration was successfully managed with chest therapy, collagen injection, and temporary gastrostomy in 32, 3, and 18 patients, respectively. Grade 1 or 2 aspiration was statistically less likely when repositioning of the pyriform sinuses was performed intraoperatively (p = .05, Table 4).

Pneumonia from aspiration (grade 3) occurred in 11.5% (14/122) of patients. None of the variables under analysis was statistically related to the inci-

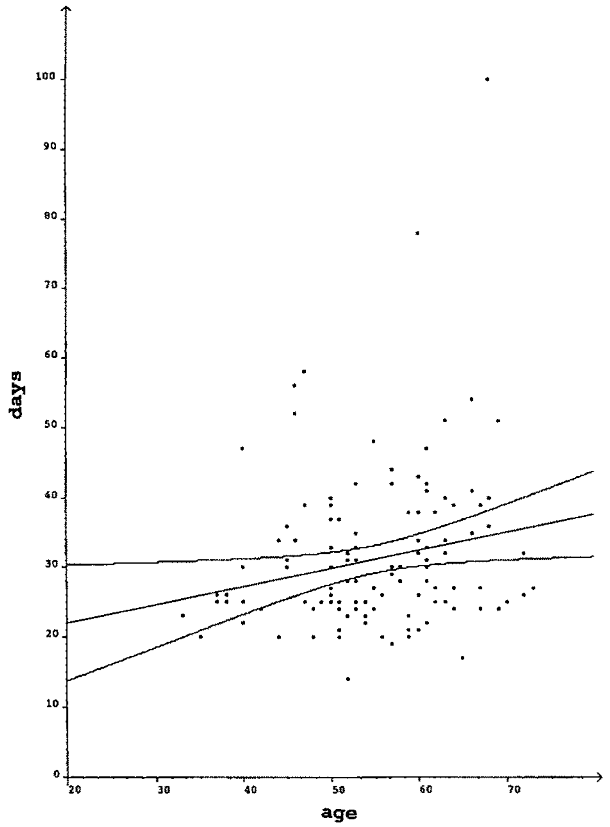

Fig 2. Length of hospitalization versus age (122 patients, Pearson's correlation).

dence of pneumonia from aspiration. Successful management with chest therapy and oral antibiotic treatment was achieved in 8 patients. Permanent gastrostomy and completion total laryngectomy were required in 3 and 3 patients, respectively.

Overall, 1 year after the procedure, 91% (111/122) of the patients retained normal deglutition without aspiration or weight loss. Occasional complaints of aspiration of liquids were noted in 4.1% (5/122) of patients. A permanent gastrostomy for recurrent aspiration of liquids was noted in 2.5% (3/122) of patients. Completion total laryngectomy related to severe aspiration was performed in 2.5% (3/122) of patients.

Length of Hospitalization (122 Patients). The mean length of hospitalization was 31 days (range 14 to 78 days). The average period in the hospital was prolonged in patients with complications (p = .01) and in elderly patients (p = .03, Fig 2).

DISCUSSION

Supracricoid partial laryngectomy with cricohyoidopexy has been advocated in selected transglottic and supraglottic carcinoma, usually managed either with supraglottic partial laryngectomy, RT, near-total laryngectomy, or total laryngectomy.[1-13]

The surgical mortality after SCPL-CHP has varied from 0% to 3.5% in the medical literature.[1-3,6,8,9,13] The immediate postoperative mortality was 1.6% in our series, and death was never directly related to SCPL-CHP completion. That such figures are not higher than the reported rates following SGL[17,18] suggests that SCPL-CHP completion did not increase the risk for postoperative death when compared to SGL. The surgical complications wound infection, hematoma, and postoperative bleeding were noted in 8%, 1.6%, and 3.2% of patients in our series, respectively. Such rates are not higher than the rates achieved after SGL; again, the completion of SCPL-CHP did not increase the risk for complications when compared to SGL.

The completion of SCPL-CHP resulted in resection of the whole glottis and paraglottic space. A T-shaped neoglottis between the tongue base and the remaining arytenoid cartilage is created at the time of SCPL-CHP. As the glottic sphincter has been totally removed, it is quite natural to note that in the reported series, swallowing impairment was the main functional problem after SCPL-CHP.[1-13] Temporary grade 1 and 2 aspiration was noted postoperatively in 43.4% of patients in our series. Successful management of grade 1 and 2 aspiration relied on regular outpatient chest therapy of 1 to 12 months' duration. Grade 3 aspiration was noted in 11.5% of patients in our series. Grade 3 aspiration required permanent gastrostomy and completion laryngectomy in 3 and 3 patients, respectively. Such figures and the overall 91% rate of normal swallowing achieved by the end of the first postoperative year demonstrate, in our opinion, that swallowing impairment after SCPL-CHP is mainly transient. Furthermore, our results in terms of swallowing rehabilitation are comparable with the results reported after SGL.[17-21] The rate of failure in reestablishment of adequate swallowing following SGL was reported to be 8% by Hirano et al,[17] 8.1% by Woisard et al,[18] 13% by Strijbos et al,[19] 15% by Beckhardt et al,[20] and 20% by Flores et al.[21] Aspiration after SGL required completion laryngectomy in 10.8%, 4%, and 0.9% of patients according to Flores et al,[21] Woisard et al,[18] and Herranz-Gonzáles et al,[22] respectively. Herranz-Gonzáles et al,[22] in a series of 110 patients treated with SGL, noted that 1 year after surgery 42% of the patients had occasional minor aspiration when drinking liquids, and 8% had frequent aspiration problems.

In the present study, repositioning of the pyriform sinuses and inferior constrictor muscles was the only variable to statistically reduce the risk of aspiration after SCPL-CHP (p = .05, Table 4). However, analysis of Table 4 demonstrates a trend (with the value of p being between .05 and .1) for increased aspiration as follows: 1) in patients in whom either the arytenoid cartilage was resected on the tumor-bearing side or the trunk of the superior laryngeal nerve was transected, 2) in patients with a past medical history of COPD or diabetes mellitus, 3) in patients irradiated preoperatively or postoperatively, and 4) in the elderly patient. Alajmo et al,[23] in an analysis of 15 patients, reported that SCPL-CHP in patients over 65 years of age predisposed them to severe deglutition problems. In our studies, 12.9% (16/124) of patients were older than 65 years of age. Increasing age revealed an increased delay for tracheostomy tube removal (Fig 1) and an increased length of hospitalization (Fig 2). However, the 91% rate of normal swallowing achieved at 1 year postoperatively in our series suggests that increased age alone should not be considered as a main contraindication to SCPL-CHP completion.

Transection of the superior laryngeal nerve in our studies also demonstrated a trend toward a higher incidence of swallowing impairment (Table 4). The trunk of the superior laryngeal nerve might be injured during the neck dissection or transected at the level of the epilarynx during the SCPL-CHP procedure. According to Kronenberger and Meyers,[24] sacrifice of the superior laryngeal nerve results in a sensory deficit in the ipsilateral hypopharynx and remaining supraglottic larynx that interferes with the patient's ability to sense secretions and food particles in those areas.

The use of cricopharyngeal myotomy to help improve deglutition in patients managed with SGL and SCPL-CHP is controversial. According to Tucker,[25] Kleinsasser,[26] and Thawley,[27] myotomy of the cricopharyngeal muscle at the time of SGL opens the esophageal inlet, facilitates swallowing, and prevents aspiration. Hirano et al[17] also reported that SGL associated with cricopharyngeal myotomy did not usually cause serious deglutition problems. In our series, associated cricopharyngeal myotomy at the time of SCPL-CHP did not statistically improve swallowing or reduce the incidence of pneumonia from aspiration. Previous reports by Strijbos et al,[19] Flores et al,[21] and Weaver and Fleming[28] described the same findings after SGL. However, one must note that cricopharyngeal myotomy in our series was only performed if cricopharyngeal hypertonia was noted on palpation. None of the patients who had cricopharyngeal myotomy in our series presented swallowing impairment. Therefore, at the present time in our department, the decision to perform cricopharyngeal myotomy is left to the surgeon's discretion.

Our data did not indicate significantly longer times to achievement of normal swallow in patients receiving postoperative RT when compared with patients who did not undergo postoperative RT. Such data are in agreement with the data reported by Rademaker et al,[29] who noted the same findings in patients managed with SGL.

Early removal of the tracheostomy tube, before removal of the nasogastric feeding tube, is, in our opinion, a key to successful rehabilitation of swallowing after SCPL-CHP. Previous reports summarized the causes of aspiration after tracheostomy: decreased laryngeal elevation, stasis of secretions in the upper airway and cervical esophagus due to local compressive forces exerted by the inflated cuff, desensitization of the protective cough reflex, and loss of coordination of laryngeal closure.[24,30,31] In our series, an increased delay for tracheostomy tube removal was statistically more likely with age ($p = .004$, Fig 1), laryngeal edema ($p = .001$), and preoperative COPD ($p = .006$).

On the basis of this retrospective study over a 20-year period of time with 124 patients, a successful outcome after SCPL-CHP is confirmed. The functional results in regard to respiration and deglutition are comparable with the results achieved after SGL. These encouraging functional results support, in our opinion, the integration of SCPL-CHP among the various partial laryngeal procedures available for patients with supraglottic or transglottic carcinoma. In patients who develop severe aspiration after SCPL-CHP, we advocate a percutaneous endoscopic gastrostomy before the end of the first postoperative month. Speech, swallowing, and chest therapies are continued until normal swallow is obtained.

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SAFETY OF ESOPHAGOSCOPY IN THE IRRADIATED ESOPHAGUS

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Carcinoma of the esophagus is a disease with a poor prognosis. Surgery is considered the treatment of choice. Patients who are surgically unresectable may receive radiotherapy, plus or minus chemotherapy. While this offers reasonable palliation, a significant number of patients develop benign or malignant strictures. Frequent esophagoscopy with dilation is required if they are to swallow. Patients with strictures or malignancy of the esophagus are more prone to complications if they undergo an esophagoscopy. We examined the morbidity of esophagoscopy in a group of patients who had received radiotherapy for carcinoma of the esophagus. Over a 10-year period, 21 patients receiving irradiation for carcinoma of the esophagus were examined. All patients underwent esophagoscopy for staging and diagnosis. The tumor locations were upper (6), middle (6), and lower (9) esophagus. The T-stages were 1 (7), 2 (6), 3 (6), and 4 (2). Five patients had dilation of a malignant stricture at the time of diagnosis. Fever developed and resolved within 24 hours in 2 patients. Following completion of irradiation, a total of 83 esophagoscopies were performed (range 1 to 11 per patient). Six patients with no tumor or stricture underwent 8 esophagoscopies (3 rigid, 5 flexible). There was 1 episode of minimal bleeding and 1 fever that resolved within 24 hours. Ten patients with malignant strictures underwent 62 esophagoscopies (5 rigid, 57 flexible). There were 17 (27%) episodes of fever (all resolved within 24 hours) and 14 (22%) episodes of bleeding. Five patients with benign strictures underwent 13 esophagoscopies (2 rigid, 11 flexible). One patient had a perforation that resolved with conservative treatment, and 1 patient developed an epidural abscess 2 months following dilation. This patient is the only one that required surgical intervention and had prolonged hospitalization. Esophagoscopy of the irradiated esophagus can be performed relatively safely and excellent palliation obtained. Morbidity consisting of minimal bleeding, and fever lasting less than 24 hours, is frequent but self-limited.

KEY WORDS — cancer, esophagoscopy, palliation, radiation, strictures.

INTRODUCTION

Esophageal carcinoma is a highly virulent tumor. Prognosis, whether treatment is by surgery or irradiation, is poor, with a 2-year survival of 20% to 30% and a 5-year survival of 4% to 6%.[1,2] The inability to eradicate residual disease at the primary site, along with early systemic dissemination of the disease, results in failure to cure what is clinically a localized tumor.[3] Autopsy series have confirmed the frequent systemic nature of the disease at or shortly after initial presentation.[4,5] Patients with early-stage carcinoma of the esophagus achieve 5-year survival rates greater than 60%. However, less than 10%[3,6] of patients present with early-stage tumors. Furthermore, on presentation more than 40% of patients are not eligible for surgery and receive palliative radiotherapy.[3,6] Thus, an increasing number of patients are receiving therapy consisting of chemotherapy and irradiation.[2,3,7-9] As more patients are treated this way, the number of patients undergoing esophagoscopy following irradiation is increasing.

A high incidence of stricture formation has been reported in patients who have received radiotherapy to the esophagus.[2,10-13] Adequate palliation of dysphagia[12,13] has been reported with repeat esophagoscopies in this patient population. We undertook to examine the incidence and types of complications in patients who had received full-course radiotherapy for carcinoma of the esophagus and undergone esophagoscopy.

PATIENTS AND METHODS

A retrospective review of the 85 patients evaluated at West Virginia University Hospitals for carcinoma of the esophagus between January 1985 and January 1995 was undertaken. Thirty patients did not receive irradiation, while 55 patients were given a full course of irradiation. Twenty-eight of these patients received irradiation postoperatively and were not included in this study. Of the remaining 27 patients, 6 patients did not have any follow-up at West Virginia University and were excluded from analysis. Twenty-one patient records (hospital and outpatient) were examined in full. These 21 patients make up the basis of this report.

Prior to treatment, patients underwent esophagos-

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TABLE 1. LOCATION IN ESOPHAGUS OF TUMORS TREATED BY RADIOTHERAPY

Tumor Location	*No. of Patients*
Upper	6
Middle	6
Lower	9
Total	21

TABLE 2. STAGE OF TUMORS TREATED BY RADIOTHERAPY

T-Stage	*No. of Patients*
1	7
2	6
3	6
4	2

copy for both staging and diagnosis. All patients also had at least 1 posttreatment esophagoscopy. Other indications for esophagoscopy after radiotherapy included dysphagia, stricture, recurrence, nausea, and vomiting. The method of esophagoscopy (fiber-optic versus rigid) and the number of esophagoscopies per patient, with the time interval between them, were noted. Stricture formation, size of opening, and recurrence of tumor were determined. If dilation was done, the method, as well as the maximum diameter of the bougies passed, was recorded. Complications consisting of fever, bleeding, abrasion, mucosal tear, perforation plus or minus mediastinitis, and whether they were recognized during esophagoscopy were noted.

RESULTS

Eighteen patients were male and 3 patients were female. Ages ranged from 42 to 81 years with a mean of 71 years. Stage of the tumor and site of the tumor can be seen in Tables 1 and 2. Sixteen patients had flexible esophagoscopy, while 5 had rigid esophagoscopy prior to definitive treatment. Six patients had dilation of a malignant stricture at the time of their diagnosis. Complications consisting of fever that lasted less than the 24 hours were seen in 2 patients (1 with a stricture that was dilated, and 1 who only received a biopsy).

Results of radiotherapy are in Table 3. A total of 83 esophagoscopies were performed (range 1 to 11 per patient). Six patients had no local recurrence or stricture formation following irradiation. These 6 patients underwent 8 esophagoscopies (3 rigid, 5 flexible). There was 1 episode of minimal bleeding, and 1 patient developed a fever that resolved within 24 hours. No other complications were reported (Table 3).

Five patients developed benign strictures after irradiation. Progressive dysphagia was the predominant symptom. These patients underwent 13 esophagoscopies (2 rigid, 11 flexible). Two had 1 endoscopy with dilation, 1 had 3 endoscopies with dilation, and 2 had 4 endoscopies with dilation. One patient who had undergone a second course of radiotherapy developed a *Pseudomonas* epidural abscess 2 months following her esophagoscopy. This was secondary to a chronic esophageal perforation and required surgical intervention. A second patient was noted to have a mucosal tear at the time of her esophagoscopy and developed cervical emphysema that resolved with conservative treatment. The remaining 3 patients had no complications (Table 3).

A malignant stricture developed in the remaining 10 patients. These patients underwent a total of 62 esophagoscopies (5 rigid, 57 flexible). Transient elevations of temperature that resolved within 24 hours occurred in 14 (22%) esophagoscopies. A small amount of bleeding was noted after 17 (27%) esophagoscopies. There was no mortality associated with this procedure.

DISCUSSION

Carcinoma of the esophagus has a tendency to present at a late stage.[2,3,6,8] Because the esophagus is a hollow and distensible organ, tumors can grow to a considerable size before causing a significant degree of dysphagia. The progressive dysphagia is what prompts the patient to seek medical help. Control of the dysphagia is the single most important goal of treatment.

Treatment of esophageal carcinoma is usually by surgery.[3,6] Unfortunately, a significant number of patients are not surgical candidates. These patients are treated by radiotherapy plus or minus chemotherapy.[3,6-9] After completing their radiotherapy, most

TABLE 3. POSTIRRADIATION STATUS OF ESOPHAGUS WITH NUMBER OF ESOPHAGOSCOPIES AND THEIR ASSOCIATED COMPLICATIONS

Postirradiation Results	*No. of Patients*	*No. of Esophagoscopies*	*Complications*		
			Fever	*Bleeding*	*Perforation*
No recurrence, no stricture	6	8	1 (16.7%)	1 (16.7%)	0
Benign stricture	5	13	0	0	2 (15.4%)
Malignant stricture	10	62	14 (22%)	17 (27%)	0
Total	21	83	15 (18%)	18 (21%)	2 (2.4%)

patients will continue to have symptoms of dysphagia. In our series of 21 patients, treated over 10 years, all patients required at least 1 esophagoscopy after completing treatment. Six patients had a single esophagoscopy for minimal symptoms to evaluate tumor response. All 6 patients had no evidence of tumor. Four of them had normal esophagi and 2 had strictures that were dilated. These 6 patients did not require further treatment. The remaining 15 patients underwent between 2 and 11 esophagoscopies (mean of 4). Two patients with persistent dysphagia had no stricture or tumor. The remaining 13 patients had strictures.

The incidence of esophageal strictures induced by radiotherapy for esophageal cancer is reported to range from 25% to 67%.[10,11,13] Strictures may develop as a result of fibrosis, from residual or recurrent cancer, or from a combination of the two. O'Rourke et al[13] reported 80 patients treated with radiotherapy alone. Thirty percent of their patients developed benign fibrotic strictures and required a mean of 2.4 dilations for symptoms of dysphagia. Six patients in our series (29%) maintained local control with no stricture formation on follow-up. Two of these patients required a second esophagoscopy for minimal symptoms of dysphagia. Fifteen patients (71%) developed strictures. Sixty-six percent (10 patients) of these strictures were due to persistent malignancy. Patients with malignant strictures were more symptomatic and required a mean of 4 dilations each. Patients with benign strictures required a mean of 2.6 dilations. Patients in our study and that of O'Rourke et al[13] who developed a benign stricture following radiotherapy maintained a reasonable quality of life. Three quarters were able to tolerate a soft to normal diet between dilations.

The major morbidity in the entire group occurred in 2 patients with benign strictures. One patient had undergone a second course of radiotherapy. She had dilation of her esophagus for severe stenosis. While her swallowing function was improved, she presented with an epidural abscess 2 months later. Radiology demonstrated a chronic perforation. She recovered after neurosurgical intervention and repair with a pedicled myogenous flap. The other patient, with a pretreatment T4 postcricoid lesion that involved the cervical spine (cured by chemoradiation), had an overaggressive dilation of her cervical stenosis. A mucosal tear was noted at the time of the dilation, and cervical emphysema subsequently developed. This resolved with conservative medical treatment.

Perforation of the esophagus is the most common serious complication of esophageal dilation. It is reported as occurring in up to 1 in 400 dilations.[14] Perforation rates from esophageal dilation have dropped from a high of 80 per 1,000 cases with rigid endoscopes to approximately 5 per 1,000 cases with flexible endoscopes.[15] Careful dilation under fluoroscopic control yields a perforation rate of less than 0.1%.[12,14] Swaroop et al[12] examined 103 consecutive patients undergoing dilation of esophageal strictures following radiotherapy for cancer of the esophagus. In their series, 1 patient suffered an esophageal perforation, while 1 other patient had a suspected esophagogastric perforation. Two patients developed unexplained fever, and there is no comment concerning the incidence of bleeding. Comparison between our study and that of Swaroop et al[12] is difficult. Our incidence of major complications was 2 of 87 (2.3%), which is within the reported range. Both patients who developed perforation were dilated with rigid endoscopes and had firm stenotic cervical lesions.

Bacteremia is a well-recognized complication of some endoscopic procedures.[16,17] Two studies have shown a 50% to 100% rate for detection of bacteremia following esophageal dilation.[18,19] The clinical significance of the bacteremia is not known. In one study, 17 volunteers without esophageal disease were dilated. None of these patients developed bacteremia. The transient elevation of temperature encountered in our patients is most likely secondary to a transient bacteremia. This was seen in 15 (18%) esophagoscopies. All patients had return of their temperature to normal within 24 hours, and 1 developed metastatic infection. We do not routinely administer antibiotics during or after the procedure unless an indication such as prosthetic heart valves, prosthetic orthopedic devices, etc, exists.

The next most frequent complication encountered during esophagoscopy is bleeding. This bleeding is mild, stops spontaneously, and is relatively common.[14,15] Major hemorrhage is only encountered when patients with achalasia have their stricture dilated.[15] Irradiation of the esophagus can cause fibrosis and variable scarring. Malignant strictures are usually ulcerative or necrotic. Passage of the esophagoscope through these areas will lead to small abrasions of the mucosa with a small amount of bleeding. It is our observation that this is a relatively common but benign occurrence. The combination of recurrent disease and a tight stricture also predisposes to episodes of bleeding during esophagoscopy. In no patient was intervention of a medical or surgical nature required to treat this complication.

SUMMARY

Patients with carcinoma of the esophagus who receive radiotherapy have been divided into three groups. There are patients who achieve local control of their tumor and do not develop strictures. These patients require 1 to 2 esophagoscopies and have very little morbidity associated with them. The second group of patients are those in whom radiotherapy produces local control of the tumor, but a stricture ensues. These patients require an average of 2.6 dilations to achieve good resolution of their symptoms. The morbidity, other than fever and minimal bleeding, is minimal. The final group of patients is those who develop a malignant stricture of the esophagus. In our series this occurred in 50% of patients. These patients can achieve excellent palliation with repeat esophagoscopies and dilation. The rate of morbidity (38%) is quite high, but in light of its minor nature, is of little consequence.

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COMPLICATIONS ASSOCIATED WITH 327 FOREIGN BODIES OF THE PHARYNX, LARYNX, AND ESOPHAGUS

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We intended to identify the types and incidence of complications associated with foreign bodies (FBs) impacted in the upper aerodigestive tract (UADT) and to ascertain factors predisposing to the development of these complications. The design was a retrospective cohort study of 327 patients with UADT foreign bodies admitted to a tertiary care center. The overall incidence (7.6%) and types of complications varied by age. Complications developed in 4.8% of 208 patients 10 years of age and under, with pulmonary complications being most common. In contrast, complications occurred in 12.6% of 119 older patients, with retropharyngeal abscess being the most common ($p < .0001$). Delayed presentation (>24 hours after the onset of symptoms) was the only factor associated with an increase in the incidence of complications in the younger patients ($p = .02$). In contrast, pharyngeal location of the FB ($p = .0004$), the FB's being a fish bone ($p = .006$), and radiolucency ($p = .02$) were all associated with an increased incidence of complications in patients over 10 years of age. A significant risk for complications is present for patients admitted for the management of FBs in the UADT. Older patients with sharp FBs are at greatest risk. In this group of patients, close observation in the perioperative period is required, especially if there is evidence of mucosal injury.

KEY WORDS — aspiration, complications, foreign body, retropharyngeal abscess.

INTRODUCTION

The processing of ingested materials is the primary function of the oral portion of the upper aerodigestive tract (UADT). Occasionally, large substances prematurely escape the oral cavity and enter the remainder of the alimentary system. The majority of these foreign bodies (FBs) pass undetected into the stool. Rarely, FBs can become impacted somewhere along the aerodigestive tract, posing a significant risk for complications. We examined our experience with FBs in the UADT in order to describe the incidence and types of complications seen in these cases and to identify factors associated with their development.

METHODS

The medical data of all patients admitted to Kings County Hospital Center for the management of an impacted UADT FB over a 10-year period ending July 31, 1995, were reviewed. For each case, the demographic data (age, sex), information regarding presentation (duration of symptoms, type of FB, underlying medical problems), location of the FB, treatment (need for operating room, method of removal), postoperative care (time to first feeding, symptoms of complication, prophylactic antibiotic use), and acute complications (type, treatment, outcome) were evaluated. The patients were divided into two groups based on age. Group 1 includes patients 10 years of age and younger. Group 2 includes all patients older than 10 years.

Statistical significance was defined as a two-tailed p value of $\leq .05$. Fisher's exact test was performed with StatXact statistical software for exact nonparametric inference.[1] All other analyses were performed with True Epistat software for medical statistics.[2]

RESULTS

During the study period, 323 patients with adequate documentation were identified for inclusion in the study. Three patients had a history of mental retardation, and multiple admissions for FBs in the UADT. In these cases, each admission was considered a separate event (total 327 events). There were 189 males (57.8%) and 138 females (42.2%) ranging in age from 4 months to 83 years, with a median age of 3 years. Two-hundred eight patients (63.6%) 10 years of age or younger were included in group 1. One hundred nineteen patients (36.4%) older than 10 years were included in group 2.

The majority of patients (93%) had a clear history of FB ingestion and all had symptoms to suggest the presence of an FB. The duration of symptoms prior to presentation was similar in both groups, with the majority of patients (72.3%) presenting within 24 hours of FB ingestion. Delayed presentation (>1

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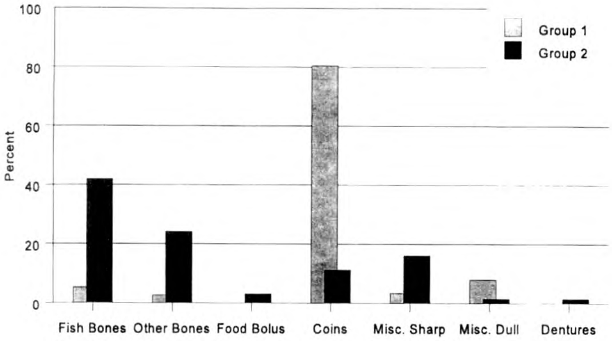

Fig 1. Type of foreign body by group (N = 327; p < .0001). Group 1 — 10 years and younger, group 2 — older than 10 years.

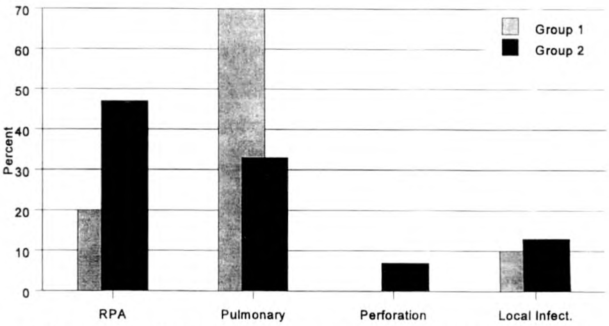

Fig 2. Complications by group (N = 25; p = .007). RPA — retropharyngeal abscess; pulmonary — aspiration, pneumonia, pneumonitis, pulmonary collapse; local infect. — esophagitis, cellulitis, ulceration.

week after onset of symptoms) was seen in only 5.4% of cases.

Radiologic examination was documented as part of the initial evaluation in 297 of 327 cases, with radiopaque FBs recorded in 243 (81.8%) of them. Foreign bodies were detected more often by radiographic examination in group 1 patients (188 of 205 patients or 91.7%) than in group 2 (55 of 92 patients or 59.8%; Fisher's exact test; $p < .00001$). Animal bones, mainly fish bones, were radiolucent in 48% of cases. In contrast, all other types of FBs were radiolucent in less than 25% of cases (Fisher's exact test; $p < .0001$). The addition of computed tomography was able to detect fish bones not seen on plain film in 3 of 7 cases (42.9%).

The anatomic location of FBs was similar in both groups, with proximal esophageal FBs (73.3%) being most common overall, followed by FBs in the larynx (8.9%), distal esophagus (8.3%), pharynx (8.3%), and oral cavity (1.2%). However, the types of FBs recovered differed between the groups, with coins being most common in group 1 patients, and fish bones in group 2 patients (Fig 1; Fisher's exact test; $p < .0001$). Factors predisposing to the development of complications were more common in the older population, occurring in 12 patients (12.6%), compared to 3 patients in the younger group (1.4%; Fisher's exact test; $p < .0001$).

Overall, complications developed in 25 of 327 of cases (7.6%), with an incidence of 4.8% in group 1 and 12.6% in group 2 (Fisher's exact test; $p = .02$). The types of complications differed between the two groups, with pulmonary complications (aspiration, pulmonary collapse, bronchitis, pneumonitis or pneumonia) most common in group 1 patients and retropharyngeal abscess (RPA) most common in group 2 patients (Fig 2; $p = .007$). No deaths occurred in either group.

Pulmonary complications mainly developed because of aspiration either during or shortly after FB removal (mainly coins), especially in the younger patients. Retropharyngeal abscess occurred exclusively in patients with sharp FBs (Fig 3; $p < .0001$). Evidence of mucosal injury or intraluminal blood was evident at the time of removal in 5 of 7 cases of RPA.

When the entire cohort was evaluated, no increased risk for complications based on sex, sharpness of the FB, or modality of removal was found. However, presentation after 24 hours ($p = .05$), pharyngeal location ($p < .0001$), radiolucency ($p = .02$), and age over 10 years ($p = .02$) positively correlated to the incidence of complications. To control for the confounding effects of age on complication development, we examined the data for each group. In group 1, only presentation after 24 hours was associated with a higher incidence of complications (Table 1). In group 2 patients, pharyngeal FBs, fish bones, FBs with sharp projections, and radiopaque FBs had a higher incidence of complications (Table 2).

DISCUSSION

Foreign body impaction in the UADT has been a problem since the earliest of reported history.[3] Technologic advancements have allowed us to master the removal of FBs. However, in spite of successful removal, complications still occur.[4] Pulmonary com-

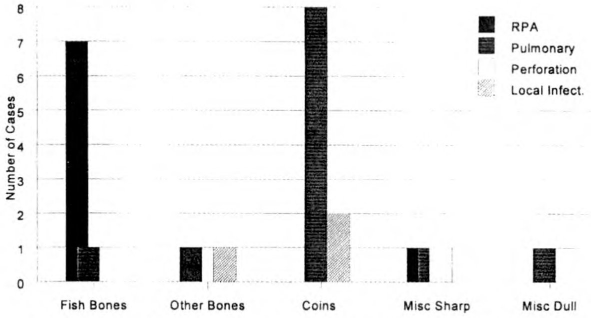

Fig 3. Complications by type of foreign body (N = 25; $p < .0001$).

TABLE 1. FACTORS ASSOCIATED WITH INCREASED INCIDENCE OF COMPLICATIONS FOR GROUP 1 (10 YEARS AND YOUNGER) PATIENTS (N = 208)

	Complication	*No Complication*	*%*	*p**
Gender (F/M)	5/5	86/112	5.5/4.2	NS
Predisposing factors (+/–)	1/9	2/196	33.3/4.4	NS
Duration of symptoms (<24 h/>24 h)	4/6	148/50	2.6/10.7	.02
Location (esophagus/other)	4/6	153/45	2.5/11.7	NS
Type of foreign body (coin/other)	6/4	155/43	3.7/8.5	NS
Sharp projections (+/–) (N = 205)	3/6	22/174	12.0/3.3	NS
X-ray detection (+/–) (N = 205)	8/1	180/16	4.3/5.9	NS
Operative removal (+/–)	6/4	157/41	3.7/8.9	NS

NS — not significant (p > .05).
*Fisher's exact test.

plications are most common, followed by RPA and local infectious complications. An increased risk for the development of these complications is associated with delayed presentation, underlying predisposing factors, fish bones, pharyngeal location, and radiolucency of the FB (Tables 1 and 2). The types and incidence of these complications vary significantly by the age of the patient at presentation.

Young patients (group 1) accounted for the majority of our study population (approximately 64%) and had a significantly lower incidence of complications. This can be explained by several differences between young and old patients. First, the most common FBs in young patients were blunt (88%; mainly coins), while sharp FBs (82%; mainly fish bones) were most common in older patients (Fig 1). In addition, the FBs recovered in older patients were more often radiolucent, potentially delaying removal. Finally, conditions that may be associated with a higher risk of complications, including mental retardation, incarceration, esophageal stricture, diabetes, and human immunodeficiency virus infection, were also more common in older patients. Although denture use was not evaluated in our study population, this may also be a risk factor for the development of complications.

Young patients are prone to complications involving the pulmonary system (aspiration, pulmonary collapse, bronchitis, pneumonitis or pneumonia). These types of complications accounted for 70% of all complications in young patients, compared to 33% in older patients. Pulmonary complications developed at the time of, or shortly after, FB removal, and were not influenced by the type of FB present. The single factor associated with an increased risk for these complications in younger patients was presentation over 24 hours after the onset of symptoms (Table 1). This suggests that early removal, under controlled conditions, should be contemplated in all cases, with a limited role given to observation.

The best modality of removal of UADT FBs has been a subject of much controversy, especially in children.[5-7] In our study, we found no increased risk for complications based on the method of removal (rigid or flexible endoscopy). However, we believe that the most important consideration in the management of children is airway control, and therefore we prefer general endotracheal anesthesia at the time of removal. Once the airway is controlled, any modality allowing direct visualization of the FB may be used for removal.

In our study population, the most common complication in patients over 10 years of age was RPA. This is different from what is reported in the literature. In an extensive review of the world literature, Remsen

TABLE 2. FACTORS ASSOCIATED WITH INCREASED INCIDENCE OF COMPLICATIONS FOR GROUP 2 (OLDER THAN 10 YEARS) PATIENTS (N = 119)

	Complication	*No Complication*	*%*	*p**
Gender (F/M)	5/10	42/62	10.6/13.9	NS
Predisposing factors (+/–)	4/11	8/96	33.3/10.3	NS
Duration of symptoms (<24 h/>24 h)	10/5	76/28	11.6/15.1	NS
Location (pharynx/other)	6/9	5/99	54.5/8.3	.0004
Type of foreign body (fish bone/other)	8/7	20/84	28.6/7.7	.006
Sharp projections (+/–) (N = 108)	9/5	80/14	10.1/26.3	.06
X-ray detection (+/–) (N = 92)	4/9	51/28	7.3/24.3	.02
Operative removal (+/–)	10/5	85/19	10.5/20.8	NS

NS — not significant (p > .05).
*Fisher's exact test.

et al[4] identified only 10 cases of RPA associated with penetrating FBs. Since then, only 28 additional cases of FB-associated RPA have been reported, the majority of which were in a single report from Singapore.[8,9] However, in previous studies we found that FBs are the most common cause of RPA in adults in our population.[10] Invariably, RPA developed because of a sharp FB, with evidence of penetrating injury detected in the majority of cases. In our population, like that in Singapore, fish bones are the most common cause of this type of complication.[9]

The increased incidence of RPA associated with fish bones compared to other animal bones may be related to the fact that fish bones can be missed radiographically, since they are often composed of cartilage. Radiolucency was associated with a significant increase in complications in adults. Computed tomographic scanning or endoscopy should be performed where fish bone impaction is strongly suspected, even if the findings on examination and radiographs are normal.

Cases of RPA were usually present at the time of presentation (63%). However, it is important to note that RPAs developed after FB removal in 3 cases (37%), all occurring within 3 days of FB removal. In 2 of these cases, evidence of mucosal injury was detected at the time of FB removal.

Several observations of this study deserve comment. It describes rates and types of complications encountered in the management of FBs in the UADT. However, being a retrospective study, it is limited to the data available in the medical records. The study is skewed by the fact that many cases of FBs in adults (mainly with bones) were managed on an outpatient basis, while younger patients more often required admission for their treatment. This may account for the higher rate of complications in older patients, but does not explain the differences in the types of complications that occurred.

On the basis of our data, we recommend early removal of impacted FBs in the UADT. At the time of removal, rapid induction anesthesia should be performed, and the stomach suctioned after FB removal to reduce the risk of aspiration. Sharp FBs associated with mucosal injury should be given special consideration. Prolonged observation is indicated in this circumstance due to the risk of developing delayed complications.

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ANALYSIS OF PAIN AND ENDOSCOPIC SINUS SURGERY FOR SINUSITIS

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This prospective clinical descriptive study was designed to see whether patients who had endoscopic sinus surgery for sinusitis had relief of pain, and whether they had new pain postoperatively. The sample was 252 consecutive patients who underwent endoscopic sinus surgery after presenting with inflammatory sinus disorders meeting specific clinical definitions of sinusitis and criteria for surgically treatable sinus disorders developed by the treating surgeon. Of the 252 consecutive endoscopic sinus surgery patients, 106 (42%) had no preoperative pain and 146 (58%) patients had preoperative sinus pain. At 6- to 12-month postoperative evaluations, patients with no preoperative pain did not develop any new postoperative pain. Among the 146 patients with preoperative pain, 82 (56%) had no pain, residual symptoms, or further sequelae, and were considered cured; 42 (29%) reported a marked improvement of pain or discomfort; 9 (6%) had the same degree of pain or discomfort as before; 3 (2%) reported worse pain or discomfort; and 10 (7%) reported new pain or discomfort. These results suggest that the application of consistent definitions and clinical criteria for various forms of surgically treatable sinusitis will more likely predict improvement of pain and discomfort following surgical treatment. The risk of developing new pain and discomfort following endoscopic sinus surgery in individuals without preoperative pain or discomfort is negligible. The risk of worsening pain or new pain was less than 10% in patients with preoperative pain. Ongoing efforts such as the International Conference on Sinus Disease in the development of consistent terminology, staging, and therapy, and future modifications from additional clinical experience, should result in more predictable and effective care in the surgical treatment for pain of sinus disease.

KEY WORDS — endoscopic sinus surgery, sinus pain, sinusitis.

MATERIALS AND METHODS

The focus of this prospective clinical descriptive study was the relief of pain, or the development of pain postoperatively, in 252 consecutive patients operated on endoscopically for inflammatory sinus disorders from January 1992 through April 1995. All patients gave informed consent for surgery and informed consent for general anesthesia. All patient evaluations, definitions for sinusitis, determinations of specific clinical criteria for surgical sinusitis, and endoscopic surgeries were performed by the same surgeon (S.D.S.), and data collection and entry were done by an experienced registered nurse. Results were then analyzed.

The term "pain" was used broadly to include any pressure or discomfort in the facial area, and was graded by the patient as no pain, worse, same, better, new, mild, moderate, or severe.

The signs and symptoms of sinusitis are those accepted in other studies.[1-3] The diagnosis of sinusitis was made with purulent discharge from the nasal passages or nasopharynx; intermittent or persistent fever; intermittent or persistent facial pain; smell or taste disorder; tenderness over the maxillary, ethmoid, or frontal sinuses; and computed tomography (CT) evidence of sinuses partially or totally opacified and blocked ostiomeatal complexes. Criteria for endoscopic surgery for sinusitis included the above and a patient history of common symptoms for sinusitis lasting greater than 6 months and unresolved with prolonged or repeated medical therapy; anterior endoscopic findings of nasal polyps or blocked ostiomeatal complexes; and/or CT evidence of disease. Some patients with signs and symptoms of sinusitis and found to have large polyps, mucoceles, or pyoceles at the time of initial examination were judged not to require the criterion of 6 months of medical therapy, and were considered endoscopic surgical candidates. The distinctions between various types of sinusitis were as follows: acute sinusitis, less than 1 month; subacute sinusitis, between 1 and 3 months; chronic sinusitis, over 3 months; recurrent acute sinusitis, no sinusitis symptoms between attacks; and chronic sinusitis with acute exacerbations, symptoms all or most of the time, with flaring of symptoms during exacerbations.

Patients were considered surgical candidates when medical therapy (usually for a minimum of 6 months) failed to provide adequate relief of their symptoms.

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TABLE 1. PAIN AS ONLY PRESENTING PREOPERATIVE SYMPTOM

- 11 patients total
- 4 patients with mucocele-pyocele
  - 2 patients with maxillary lesion
  - 2 patients with frontal lesion
- 4 patients with complications of previous sinus surgery
  - 2 patients with stenotic nasofrontal ducts
  - 1 patient with stenotic middle meatal antrostomy
  - 1 patient with posterior ethmoid mucocele
- 1 patient with glabellar pain; acute sphenoid sinusitis refractory to treatment
- 1 patient with bilateral maxillary and frontal pains; polyps in middle meati
- 1 patient with unilateral frontal pain when flying; large ipsilateral agger nasi cell was opened

When a significant imaging abnormality was discovered incidentally (mucocele with expansion; a totally opaque frontal, maxillary, or sphenoid sinus; chronic maxillary atelectasis; ossifying fibroma of the maxilla) or when a fungal involvement was suspected on CT (fungus ball or allergic fungal mucin), the patients were considered surgical candidates even without a trial of medical therapy.

RESULTS

Of the 252 consecutive endoscopic sinus surgery patients, 106 (42%) had no preoperative pain and 146 (58%) patients had preoperative sinus pain. At 6- to 12-month postoperative evaluations, patients with no preoperative pain did not develop any new postoperative pain. Among the patients with preoperative facial sinus area pain, 110 (75%) patients had bilateral pain, 36 (25%) had unilateral pain, 114 (78%) had recurrent pain, and 32 (22%) had constant pain. In 135 (92%) patients, the pain was associated with one or more other rhinologic symptoms (nasal obstruction, congestion, anterior and/or posterior discharge, smell and taste disorder). In 11 (8%) patients, pain was the only symptom, but there were clinical or CT signs of disease that were considered to require surgical attention and are summarized in Table 1.

TABLE 2. PREOPERATIVE DIAGNOSES

	%
Acute sinusitis	1
Recurrent acute sinusitis	17
Chronic sinusitis	56
Subacute sinusitis	1
Chronic with acute sinusitis	9
Mucocele-pyocele	6
Complication of sinusitis	1
Polyps	21
Recurrent polyps	18
Imaging abnormality	8
Atypical facial pain	12

Note that total of percentages is greater than 100%, as many patients had more than one diagnosis.

TABLE 3. PAIN DESCRIPTION

	No.	%
Pressure alone	108	74
Pressure and pulsating	17	12
Pressure and stabbing	9	9
Pulsating alone	5	5
Pulsating and stabbing	1	1
Stabbing alone	5	5
Pressure, pulsating, and stabbing	1	1

Seven (29%) of those patients with pulsating element to their pain were judged to have additional migraine headache.

Table 2 summarizes the diagnoses assigned to the patients. Many patients had more than one diagnosis.

Table 3 summarizes the descriptions of the pain. Pressure pain alone was most common, and 7 (29%) of the 24 patients reporting a pulsating characteristic to their pain were also found to suffer from migraine headaches. In some cases, sinusitis attacks were observed to trigger migraine headaches.

The pain varied in intensity and was usually repetitive. Thirty-six (25%) patients had pain that was mild, tolerable, and required no treatment. Eighty-seven (60%) patients described their pain as moderate and responding to over-the-counter medications. For 23 (16%) patients, prescription analgesics were required. The pain was most frequently experienced over the specific sinus involved. The pain radiated to the vertex in 16 (11%) patients, to the occiput in 8 (5%) patients, to the temporal area in 4 (3%) patients, and to the parietal skull in 3 (2%) patients.

Table 4 describes the 6- to 12-month postoperative evaluations. Among the 146 patients with preoperative pain, 82 (56%) had no pain or other sinus symptoms and were considered cured; 42 (29%) reported a marked improvement of pain or discomfort; 9 (6%) had the same degree of pain or discomfort; 3 (2%) reported worse pain or discomfort; and 10 (7%) reported a new pain or discomfort. The postoperative pain in noncured patients was accompanied by signs and symptoms of sinusitis.

TABLE 4. SIX- TO TWELVE-MONTH POSTOPERATIVE EVALUATIONS OF 146 PATIENTS WITH PREOPERATIVE PAIN

	No.	%
No pain	82	56
Better	42	29
Same	9	6
Worse	3	2
New	10	7

TABLE 5. NEW PAIN AT SIX- TO TWELVE-MONTH POSTOPERATIVE EVALUATIONS

Of 146 patients with preoperative pain, 10 patients developed new postoperative pain	
4	Not bothersome; no therapy needed
3	Related to recurrence of infections
1	New maxillary pain; negative examination; found to have allergic fungal sinusitis
1	Glabellar pain; drill used for frontal sinusotomy
1	Related to development of ethmoid mucocele

The 10 patients with preoperative pain who developed new postoperative pain are summarized in Table 5. In 1 patient, the new pain was due to a postoperative ethmoid mucocele. Another patient's new glabellar pain was thought to be due to the extensive use of a drill to facilitate endoscopic frontal sinusotomies.

Among patients with no preoperative pain, none developed new pain following surgery.

Of the 252 consecutive patients treated by endoscopic sinus surgery, 30 (12%) had pain that could not be completely explained by the sinus disorders, and were referred to a multidisciplinary facial pain clinic attended by several physicians of different medical specialties, including the authors. Other diagnoses made at this clinic included myofascial pain; vascular headaches; idiopathic, posttraumatic, postsurgical, and postinfectious neuralgias; analgesic and antibiotic habituation; allergies; and dental disease involving a variety of treatment strategies. These and other patients seen in this clinic are the subject of an ongoing study.

DISCUSSION

Recent efforts to improve the care of patients with sinusitis include uniform anatomic terminology; a scoring system to quantify historical, endoscopic, and radiographic findings; and standardized guidelines for medical therapy for acute and recurrent acute sinusitis.[4] In the treatment of headache disorders, the classification system developed by the International Headache Society was developed to help ease the confusion in the classification and nomenclature of headache and facial pain syndromes; to allow for consistency in nomenclature among physicians; and to facilitate communication, research, and treatment among many clinicians around the world.[5] Clinicians from different specialties have a variety of approaches and biases in the investigation and treatment of facial, sinus, and head pains. Patients also have biases, particularly when convinced they have sinusitis, even in the face of no supporting clinical or historical data.[6,7]

However, even data obtained from commonly accepted signs and symptoms for sinusitis are not always predictive of or diagnostic for sinusitis. A recent study in the *British Medical Journal* concluded that the clinical history and examination are essentially worthless in the diagnosis of acute maxillary sinusitis.[7] In patients with clinical suspicion for acute maxillary sinusitis referred from eight general practices in Denmark, and in whom sinusitis was later confirmed or excluded by antral puncture, only the erythrocyte sedimentation rate and concentration of C reactive protein were independently associated with the diagnosis of acute maxillary sinusitis. The traditionally accepted symptoms (illness of more than 1 day, previous sinusitis, preceding upper respiratory tract infection, cough, nasal congestion, maxillary pain, maxillary toothache, pain when bending forward, anosmia, cacosmia) and signs (temperature over 38°C, purulent nasal discharge, purulent pharyngeal discharge, swollen and inflamed turbinates, tenderness over the maxillary sinuses, tenderness on tapping the maxillary teeth, and edema over the maxillary sinuses) were not independently associated.[7] The Danish study and this descriptive study's findings regarding the surgical treatment of sinusitis with the focus on facial pain appear to reinforce the need for consistent application of historical and clinical criteria, and definitions for sinusitis and other facial pain disorders.[6]

This prospective clinical descriptive study presents an analysis of a prospective database that began before the publication of the two cited studies,[6,7] and focuses on sinus pain and one particular surgeon's (S.D.S.) consistent application of reasonable definitions, clinical evaluations, and clinical criteria for instituting endoscopic surgical treatment for sinus disorders. The surgeon of this study did not offer endoscopic sinus surgery for facial pain unless there was evidence to support a diagnosis of sinusitis by history, endoscopic examination, and/or CT. Evidence from history alone, anterior endoscopic examination alone, or CT alone is usually not enough to make a diagnosis of sinusitis.[7]

The term "pain" was used broadly to include any pressure or discomfort in the facial area, and was graded by the patient as no pain, worse, same, better, new, mild, moderate, or severe. We acknowledge that a commonly used method for assessing pain is the Visual Analog Scale. However, in the initial development of the prospective database, this scale was not employed, to allow patients to report any pressure or discomfort in the facial area and not be biased to report only pain. This was continued for consistency and continued ability for comparisons over the course of the study.

The diagnosis of sinusitis is made so frequently and is so readily accepted by patients that it is not always clear whether any particular patient truly has had sinusitis in the past.[6,7] A phenomenon that we have noted with our patients is that the diagnosis of sinusitis is more socially acceptable as compared to other causes of headache as a reason for excused time from the workplace. Patients feel their workplace supervisor will be more accepting of sinusitis than headache of unknown cause. Further confusion often develops with the temporal association of the administration of antibiotics and the improvement of pain, even with a lack of convincing evidence to support the diagnosis of sinusitis. This could be a placebo effect; a coincidence of headache not associated with sinusitis spontaneously resolving; or perhaps an analgesic effect on the part of some antibiotics, as an animal model suggests.[8] The possibility of vascular headache should be considered when local or oral decongestants appear to relieve pain in the absence of proof for sinusitis.[6] The possibility of a referred vascular-type headache as a result of paranasal sinus disease should also be considered, and a model explaining the possible mechanism has been described by Clerico.[9] Vertex pain, frontal headache, eye and eyebrow pain, and cheek pain are often the result of referred pain of myofascial origin. Specific muscles with active trigger points found on palpation, which are known to cause referred pain that could be confused with facial pain and headache of other causes, include the splenius capitis, splenius cervicis, sternocleidomastoid, frontalis, temporalis, masseter, occipitalis, orbicularis oculi, and trapezius muscles.[10] Local anesthetic injections of muscle trigger points can be both diagnostic and therapeutic. Other therapies may include spray and stretch, physical therapy, biofeedback, behavioral medicine including identification of physical and emotional stressors, work environment and body mechanics changes, and medications that may include nonsteroidal anti-inflammatories, analgesics, and serotonin reuptake inhibitors.

Dramatic improvements in imaging technologies (CT and magnetic resonance imaging) have provided much more information about the paranasal sinuses. The sinuses are imaged when brain scans are done, giving insight into the circumstances when the patient may have no symptoms. When there are symptoms that suggest sinusitis, the scans give essential information. Endoscopes are widely available and frequently used. The patient who may have sinusitis can be examined. The ostia of the sinuses can be examined. Endoscopes also allow surgical treatments to be discrete and focused on very specific areas. With the new information available from imaging and endoscopes, the understanding of sinusitis and its symptoms is changed. Definitions, signs, and symptoms need to be considered again. The term sinusitis is used so loosely that it is hard to know what it means. When sinus surgery is recommended as a treatment for headache or facial pain, especially when the imaging studies show normal sinuses, there is room for skepticism. Our multidisciplinary facial pain–headache clinic sees innumerable patients who have had sinus surgery for facial pain and who are no better. Careful measurement of pain before and after surgery and outcome analysis will be necessary in these patients.[11]

Pain is not usually a major focus of study in otolaryngology residencies. Neurology, psychology, behavioral medicine, and psychiatry are disciplines that provide special insights into the study of pain. A multidisciplinary approach will be necessary if there is to be a better understanding of facial pain, particularly as it might be helped by sinus surgery.

This study indicates the probable success of the adoption of an agreed proper and consistent methodology in the diagnosis of facial pain, and in particular, sinus disease that can be helped by endoscopic sinus surgery. This information is a necessary preliminary step in the next analysis: what types of pain can be attributed to specific sinuses when there is no infection. Ongoing efforts such as the International Conference on Sinus Disease in the development of consistent terminology, staging, and therapy, and future modifications from additional clinical experience, should result in more predictable and effective care in the surgical treatment for pain and discomfort of sinus disease.

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POSTERIOR CEPHALOMETRIC RADIOGRAPHIC ANALYSIS IN OBSTRUCTIVE SLEEP APNEA

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Our objective was to evaluate the relationship between posterior facial cephalometric measures and obstructive sleep apnea syndrome (OSAS). We used a consecutive sample of 60 patients with OSAS who underwent upright lateral cephalograms, uvulopalatopharyngoplasty (UPPP), and preoperative and postoperative polysomnography. Successful responders to UPPP were arbitrarily defined as having a respiratory disturbance index reduced to fewer than 20 events per hour. Standard cephalometric measurements were used. Posterior facial height measures were constructed, based on a plane perpendicular to the Frankfort horizontal placed at hyoidale. The total and lower airway lengths were shorter and posterior mandibular height was longer in UPPP responders compared to nonresponders ($p \leq .05$). There was no difference between the two groups by standard cephalometric measurements. Responders and nonresponders to UPPP have significant differences in posterior airway measures that are not reflected in standard cephalometric measures. Airway length likely is a critical factor in OSAS and surgical response.

KEY WORDS — cephalometry, obstructive sleep apnea, radiography, upper airway, uvulopalatopharyngoplasty.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a potentially life-threatening disorder principally characterized by hypersomnolence during the daytime and heroic snoring during sleep. Both cardiovascular and pulmonary complications can occur along with occupational and/or lifestyle disability. The most common therapy for OSAS is nasal continuous positive airway pressure (CPAP). Although demonstrated effective in many OSAS patients, nasal CPAP use and compliance are not always successful.[1,2] An established, but more invasive, form of treatment is the uvulopalatopharyngoplasty (UPPP).[3] Historically, a 50% response rate to UPPP has been reported according to a success criterion of 50% reduction in the respiratory disturbance index (RDI), defined as apneic plus hypopneic events per hour. It is generally accepted that a 50% reduction in RDI is inadequate to define successful treatment in most OSAS patients. A more clinically relevant criterion for success is an RDI of fewer than 20 events per hour or an apnea index of fewer than 10 events per hour.[4,5] With this definition of a clinical response, UPPP is successful in approximately one third of OSAS patients.[6]

Cephalometric radiographic analysis is one of the earliest methods used to discriminate responders from nonresponders to UPPP by identifying patients with retrolingual collapse.[7,8] This method offers the advantages of being both objective and noninvasive, along with having easy accessibility to clinicians and a relatively low cost. However, traditional cephalometric measures based on skeletal landmarks provide only an indirect assessment of the airway. Cephalometric measurements based on airway landmarks are significantly affected by phase of respiration, patient position, and radiographic technique. No study to date using skeletal landmarks has been predictive of response to UPPP.[7-10]

Instability and collapse of the soft tissue supralaryngeal airway define OSAS.[11] An anatomically small airway and loss of physiologic muscle tone are factors that predispose to collapse. Considered as a collapsible tube, the airway can become more susceptible to collapsing forces and, therefore, less stable as its length increases.[12] During its development to an adult configuration to allow speech, the airway lengthens.[13] Cranial landmarks combined with the hyoidale provide skeletal points to objectively measure airway length. The purpose of this study is to determine whether there is a difference in our ability to predict which UPPP patients will have a clinical cure by using posterior airway measurements versus traditional cephalometric measures.

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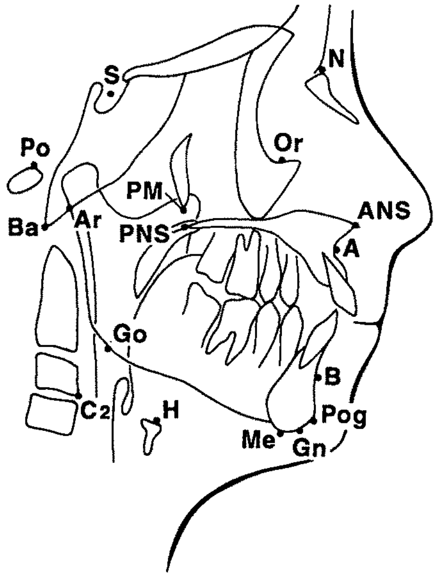

Fig 1. Cephalometric skeletal landmarks.

METHODS

Materials. The charts and cephalometric radiographs of 67 patients who had undergone UPPP at Oakland Apnea and Otology (Detroit, Mich) between 1988 and 1992 were retrospectively reviewed. Sixty-six patients were available for analysis, having met the criteria of preoperative and postoperative polysomnography along with preoperative lateral cephalometric radiographs. One patient was excluded due to a technically inadequate radiograph. Uvulopalatopharyngoplasty was performed as described by Fujita et al.[3] Nocturnal polysomnography was performed a minimum of 6 weeks following surgery and included measures of sleep (electroencephalography, electrooculography, submental electromyography), a single channel electrocardiogram, respiration (airflow and respiratory effort), and leg movements. A "responder" to UPPP was defined by an RDI reduced to fewer than 20 events per hour and a greater than 50% reduction in RDI.

Cephalometric radiographs were taken in a specially designed dynamic cephalometric apparatus (X-ray Systems, Inc, Fraser, Mich) that allowed standardized end inspiratory and end expiratory films to be taken upright and supine while maintaining a fixed position of the head to the x-ray source and to the film. Upright end expiratory films were analyzed in this study. Radiographs were hand-traced onto acetate and landmarks identified by a single trained individual who was blinded to clinical results. Acetate tracings were measured by hand. Data were entered into a computer-based spreadsheet for statistical analysis. Single analysis of variance ($p < .05$) was used to identify differences between the responders and nonresponders to UPPP.

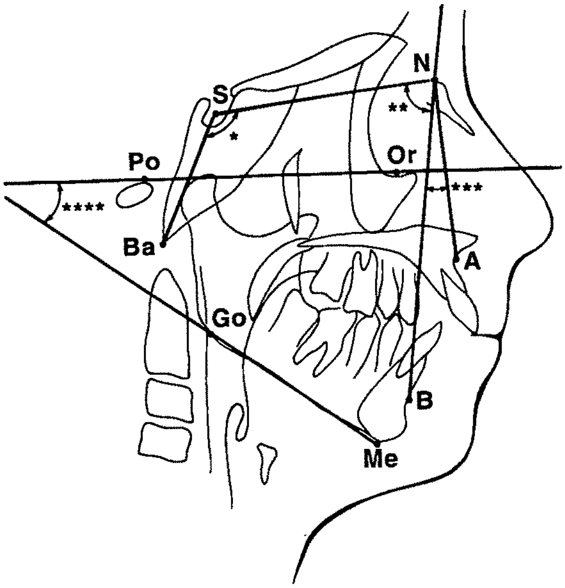

Fig 2. Constructed cephalometric angles and planes. Skull base angle Ba-S-N (*), maxillary protrusion–SNA (**), maxillo-mandibular angle ANB (***), and mandibular plane angle (****) are shown.

Measurements. Twenty-six measurements were performed on skeletal landmarks in this study (Fig 1). Twenty-one linear distances, three constructed planes, and five constructed angles were obtained (Fig 2). Measures of anterior facial projection were based on the sella (S), nasion (N), subspinale (A), and supramentale (B). Midface landmarks also included the anterior nasal spine (ANS), posterior nasal spine (PNS), and pterygomaxillare (PM). Mandibular landmarks included the articulare (Ar), gonion (Go), gnathion (Gn), and menton (Me). The hyoid position was measured by the hyoidale (H-3), the most anterior superior point on the body of the hyoid. Other skull and reference points included the base of the skull at the basion (Ba), the external bony meatus at the porion (Po), the most anterior inferior point of the atlas (C-2), and the orbitale at the inferior orbital rim (Or).

TABLE 1. CEPHALOMETRIC MEASUREMENTS

Anterior facial projection	SNA, SNB
Mandibular retrognathia or micrognathia	ANB, Ar-Pog, Go-Pog
Skull base angle	Ba-S-N
Mandibular plane (Go-Gn) measures	Mandibular plane angle (Go-Gn to Po-Or) Mandibular plane to hyoid distance (MP-H)
Width of anterior face	S-N, ANS-PNS, H3-N
Width of posterior face	Ba-PM, Ba-PNS, H2-PNS, H1-C2
Anterior facial height	N-A
Posterior skeletal height	S-Go, Ar-Go
Posterior airway length	H1-H2, H2-H3, H1-H3

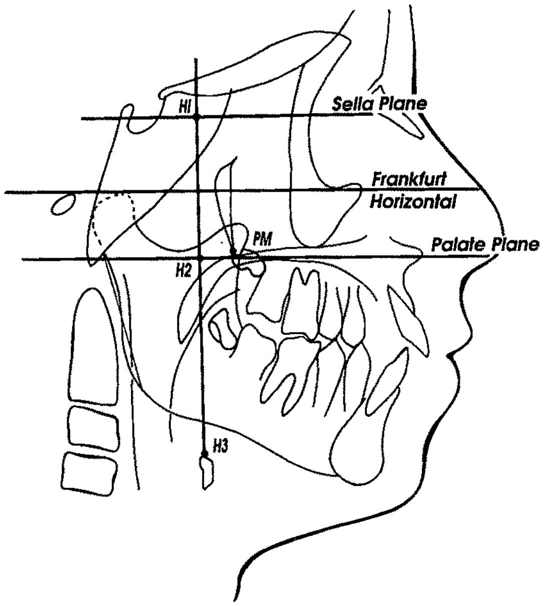

Fig 3. Constructed posterior cephalometric airway landmarks and planes.

TABLE 2. MEAN CEPHALOMETRIC DATA

	Responder	*Nonresponder*	*p*
N	32	34	NS
Sex (M/F)	29/3	32/2	NS
Age (y)	47.3 ± 10.2	49.3 ± 10.4	NS
Initial RDI	42.8	57.2	NS
Final RDI	8.3	43.4	.0001
Body mass index	30.8	32.2	NS
SNA	82.2	80.1	NS
SNB	80.6	79.5	NS
ANB	0.6	1.6	NS
Ba-S-N	128.8	127.8	NS
MP angle	26.2	24.6	NS
S-N	75.6	76.1	NS
S-Pm	21.2	21.6	NS
S-H1	15.8	16.5	NS
S-Ba	43.6	43.7	NS
S-Go	83.3	86.7	.04
Ba-PNS	49.2	50.2	NS
Ba-PM	43.4	42.6	NS
H1-H2	46.3	47.3	NS
H1-H3	124.5	130.5	.008
H1-N	60.1	60.4	NS
H2-H3	76.3	83.1	.006
H3-C2	35.4	36.2	NS
H3-POG	58.7	60.0	NS
MP-H3	26.7	29.0	NS
ANS-PNS	50.0	49.5	NS
H2-PNS	11.1	12.4	NS
PNS-sella plane	46.1	45.5	NS
Ar-Pog	119.9	122.9	NS
Ar-Go	52.2	55.5	.02
Go-Pog	83.3	84.1	NS
N-A	61.6	59.3	NS

Measures from traditional cephalometric landmarks are described in Table 1. Posterior facial height measures (Fig 3) were constructed from a plane perpendicular to the Frankfort horizontal at the hyoidale (H-3). The superior point of this line (H-1) was the intersection of this line and the constructed plane of the sphenoidale (parallel to the Frankfort horizontal). The intersection of the palatal plane and the hyoid perpendicular describes the superior extent of the soft tissue airway (H-2).

RESULTS

Of the 66 patients studied (61 males and 5 females; mean age 48.4 ± 10.2 years), the mean initial RDI for the group was 50.2 ± 32.3 events per hour. Gender, age, and initial RDI did not differ between responder and nonresponder groups. Thirty-two patients responded to UPPP and 34 were nonresponders. The results are shown in Table 2. Four measures were significantly different between the two groups. Total and lower soft tissue airway length (H1-H3 and H2-H3) were increased in nonresponders, as were two measures of posterior skeletal facial height (Ar-Go and S-Go). The SNA, SNB, and MP-H did not differ significantly between groups.

DISCUSSION

The early cephalometric radiograph analysis reports attempted to identify the OSAS patients with retrolingual collapse as defined by an abnormally low hyoid position in relation to the mandibular plane, a retruded mandible, and a narrow posterior airway space.[7,8] These initial studies found that an increased mandibular plane to hyoid distance had a correlation with UPPP failure. Ryan et al[14] used measures of inferior airway anterior-posterior dimensions and tongue volume to identify retrolingual collapse and predict UPPP response.

In several more recent studies, traditional cephalometry was used in combination with additional procedures to reduce variability and improve sensitivity. In one study, dynamic cephalograms were obtained in upright and supine positions during inspiration and expiration phases of respiration. Analysis of the resulting radiographs did not differ in any measures based on conventional skeletal landmarks or soft tissue airway width, between responders and nonresponders to UPPP. However, an anterior-posterior measure of nasal airway diameter (base of skull to posterior nasal spine) was reduced in nonresponders.[9] A subsequent prospective evaluation of standard cephalometric radiographic measurements combined with the Mueller maneuver during fiber-optic nasopharyngoscopy to assess lateral airway dimensions found no significant differentiation between UPPP responders and nonresponders.[10] All of the above cephalometric studies for prediction of UPPP response used a broad definition of clinical response (50% reduction in RDI or 50% reduction in apnea index).

In this study, posterior cephalometric airway measures were the only parameters that differed between responders and nonresponders, even with a more stringent criterion of clinical response to UPPP. Both the total vertical airway length (H1-H3) and soft tissue supraglottic airway length (H2-H3), and posterior mandibular height (S-Go, Ar-Go) were greater in the UPPP nonresponders. It is postulated that a longer pharyngeal airway has the potential for greater instability and collapse, with the UPPP procedure having a greater chance of failure.

Anterior-posterior dimensions of the airway have been demonstrated to affect response to UPPP.[5,6,12] In addition, lateral wall collapse has been shown important in OSAS.[15] However, this is the first study to indicate that airway length is a critical variable in OSAS and in response to UPPP. This study supports the concept that airway stability, in combination with airway narrowing, affects the treatment outcome in OSAS.

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DEPENDENCE OF MIDDLE EAR GAS COMPOSITION ON PULMONARY VENTILATION

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The middle ear (ME) steady state gas composition resembles that of mixed venous blood. We changed arterial and venous blood gases by artificially ventilating anesthetized guinea pigs and measured simultaneous ME gas changes during spontaneous breathing, hyperventilation, and hypoventilation. During hyperventilation, PaCO2 and PvCO2 (a = arterial, v = venous) decreased from 46.0 and 53.0 mm Hg to 17.9 and 37.5 mm Hg, respectively, while PaO2 and PvO2 (85.6 and 38.2 mm Hg) did not change. This was accompanied by an ME PCO2 decrease from 70.4 to 58.8 mm Hg and a PO2 decrease from 36.8 to 25.4 mm Hg. During hypoventilation, PaCO2 and PvCO2 increased to 56.8 and 66.4 mm Hg, while PvO2 decreased to 21.8 mm Hg. The ME PCO2 increased simultaneously to 88.8 mm Hg and the ME PO2 decreased to 25.4 mm Hg. The ME PO2 decrease during hyperventilation may be explained by a 33% decrease in ME mucosa perfusion, calculated from the ME ventilation-perfusion ratio. This study shows that ME gas composition follows fluctuations of blood gas levels and thus may affect total ME pressure.

KEY WORDS — blood gases, guinea pig, hyperventilation, hypoventilation, middle ear gases, middle ear pressure, ventilation-perfusion ratio.

INTRODUCTION

Bidirectional diffusion between middle ear (ME) and blood gases has been repeatedly demonstrated.[1-8] Nitrous oxide and halothane used in anesthesia pass from blood into the ME cavity, resulting in higher ME pressure.[1,2,3,6] Freon-22 introduced into the inspired air, bypassing the eustachian tube, was detected in the ME of guinea pigs within 8 minutes.[8] Gas diffusion in the opposite direction, from the ME into circulating blood, was also demonstrated when oxygen (O2), carbon dioxide (CO2), or nitrogen (N2) was politzerized into atelectatic MEs. Each particular gas left the ME at a rate proportional to its diffusion coefficient.[4]

Middle ear gas pressures at steady state are considered to be rather similar to mixed venous blood gas pressures.[7,9-14] It is therefore likely that dynamic changes in blood gas composition will be reflected in ME gas partial pressures. The purpose of this study was to examine the influence of dynamic blood gas changes on ME gas composition by simultaneous measurements of arterial, venous, and ME gas partial pressures during spontaneous breathing, hyperventilation, and hypoventilation.

MATERIALS AND METHODS

Experimental Animals, Anesthesia, and Surgical Procedure. Eleven female guinea pigs, mean body mass 740 g (range 500 to 1,150 g), were anesthetized by subcutaneous injection of 2% xylazine hydrochloride and 10% ketamine hydrochloride (0.05 mL/100 g body mass). In addition, local anesthesia (1% lidocaine hydrochloride) was injected subcutaneously at sites of surgical incisions: into the right retroauricular region and to the ventral aspect of the neck. An electric cushion kept the body temperature at about 37°C throughout the experiment. The right bulla was exposed through a retroauricular incision, and a 1.2-mm hole was drilled in its wall. A gas sampling probe connected to the mass spectrometer and a thermocouple were introduced into the bulla (Fig 1). The thin furrow between the probes and the bulla was sealed off with Vaseline ointment.

The trachea was exposed and transected through a midline neck incision. The proximal end of the trachea was occluded. Ventilation was maintained through a tube inserted into the distal end of the trachea. When artificially ventilated, the tube was connected to a ventilator (Fig 1). The left internal carotid artery and the right external jugular vein were exposed and a thin bypass catheter was inserted into each respective lumen (Fig 1). Blood circulating from the heart to the cranium and vice versa passed through the bypass catheter. The bypass catheters were made of thin polyethylene tubes (inside diameter 0.85 mm) connected to a closed polyethylene chamber (Fig 1). Blood samples of 80 μL were taken

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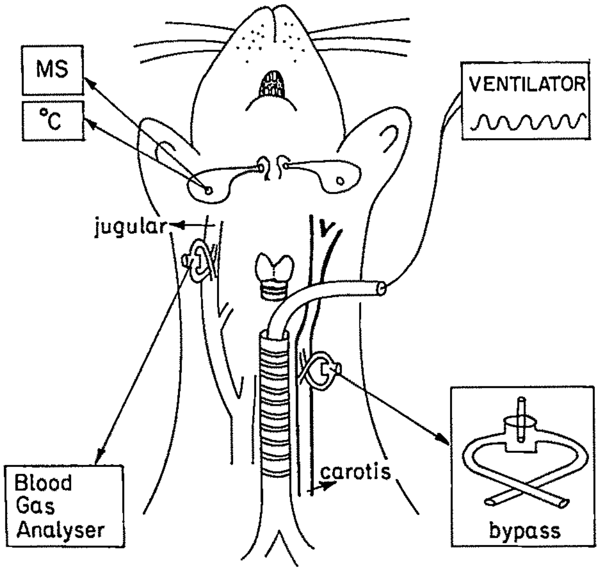

Fig 1. System used for measuring simultaneous gas changes in blood (arterial and venous) and middle ear in guinea pigs. MS — mass spectrometer (see text).

from this chamber and diluted heparin sodium (5 IU in 0.1 mL saline) was injected into it to prevent clotting. Blood samples were stored on ice until analyzed.

During experiments, animals were allowed to breathe spontaneously until repetitive (steady state, plateau) gas concentration values in the ME were reached.[14,15] Once steady state was reached, baseline duplicate blood samples were taken. The animals were then connected to the ventilator and artificially hyperventilated or hypoventilated. Hyperventilation and hypoventilation were induced for 30 to 40 minutes each, and then additional duplicate blood samples were taken.

Middle Ear Gas Measurements. The ME gas measuring system was composed of a mass spectrometer (Amis 2000) equipped with a diffusion sampling probe as described previously.[15] The sampling probe is a capillary tube with a sintered bronze tip cupped by polyethylene. Gases reach the mass spectrometer only by diffusion through the polyethylene cover. Sampling was continuous and measurements were performed once every second. Mass spectrometer gas consumption was 2×10^{-6} mL/s.[14] Data were displayed on-line and stored.[15,16]

Blood Parameters. The P_{CO_2}, P_{O_2}, pH, carbonic acid, and oxygen saturation in arterial and venous blood samples were measured by a blood gas analyzer (Radiometer, ABL500) at 37°C.

Artificial Ventilation. A small animal ventilator (M-D Industries) was used for artificial ventilation. The air was humidified before reaching the ventilator. Positive end-expiratory pressure (PEEP) of 1 cm H_2O was employed to prevent atelectasis. Ventilation volumes in the range of 210 to 920 mL/min were precalibrated with a volumeter (Brooks Instruments NV, Veenendaad, the Netherlands). For each guinea pig, an expected tidal volume (VT) was calculated according to body mass [VT(mL) = 3.97×10^{-3} body mass (g) + 2.5].[17] The spontaneous breathing rates of the anesthetized animals were measured with a stop-

MIDDLE EAR AND BLOOD GAS COMPOSITION DURING SPONTANEOUS BREATHING, HYPERVENTILATION, AND HYPOVENTILATION IN GUINEA PIGS

		Spontaneous Breathing (n = 11)	*Hyperventilation (n = 5)*	*Hypoventilation (n = 6)*
Artery (internal carotid)	PO_2 (mm Hg)	57.5 ± 11.13	58.3 ± 21.65	48.3 ± 6.59
	PCO_2 (mm Hg)	46.0 ± 4.55	17.9 ± 5.84*	56.8 ± 10.47*
	pH	7.35 ± 0.04	7.53 ± 0.21	7.31 ± 0.10
	O_2 saturation (%)	84.9 ± 9.33	85.6 ± 15.84	77.6 ± 9.37
	HCO_3^- (mmol/L)	26.33 ± 4.58	15.8 ± 6.06	27.8 ± 4.90
Vein (external jugular)	PO_2 (mm Hg)	33.2 ± 9.37	29.5 ± 13.85	21.8 ± 5.33*
	PCO_2 (mm Hg)	53.0 ± 6.66	37.5 ± 9.86*	66.4 ± 10.7*
	pH	7.30 ± 0.05	7.38 ± 0.18	7.21 ± 0.09
	O_2 saturation (%)	58.0 ± 14.06	38.2 ± 30.48	20.6 ± 6.94
	HCO_3^- (mmol/L)	26.6 ± 5.21	21.1 ± 3.73	25.4 ± 5.34
Middle ear cavity	PO_2 (mm Hg)	36.8 ± 6.17	27.3 ± 7.14*	25.4 ± 9.94*
	PCO_2 (mm Hg)	70.4 ± 11.24†	58.8 ± 7.88*†	88.8 ± 15.56*†
	Temperature (°C)	36.9 ± 0.95	37.0 ± 0.21	37.4 ± 0.50
	$\dot{V}ME/\dot{Q}ME$ ($\times 10^{-4}$)	8.6 ± 1.92	12.9 ± 1.86	7.2 ± 4.05

Data are mean ± SD.

n — number of animals, $\dot{V}ME$ — middle ear ventilation through eustachian tube, $\dot{Q}ME$ — perfusion of middle ear mucosa.

*Significant difference ($p < .05$ and higher) between spontaneous breathing and either hyperventilation or hypoventilation.

†Significant difference between middle ear and venous blood values within same experimental condition.

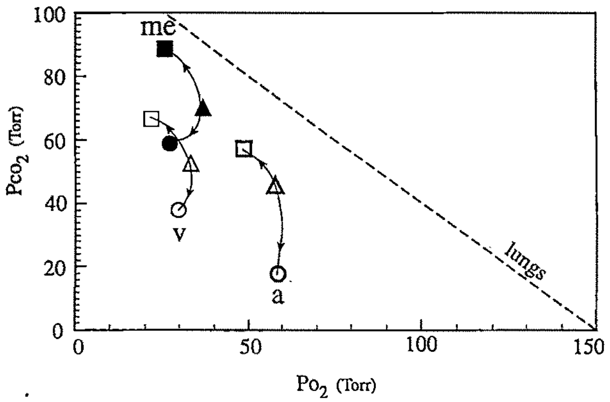

Fig 2. Mean values of CO2 partial pressure (PCO2) as function of O2 partial pressure (PO2) in arterial blood (a), venous blood (v), and middle ear (me) during spontaneous breathing (Δ; n = 11), hyperventilation (O; n = 5), and hypoventilation (□; n = 6) in guinea pigs. Data are mean values. For standard deviations, see Table. Dashed line is lungs R line (=.80).

watch and were considered as normoventilation. The spontaneous respiration rates of anesthetized guinea pigs were in the range of 50 to 80 breaths per minute. These served as a baseline for the artificial ventilation setup. In hyperventilation experiments, the ventilation rate was set to be 150% of the reference value, whereas in hypoventilation, the rate was set as 50% of the baseline rate.

Middle Ear Temperature. The temperature within the bulla was measured continuously with a copper-constantan thermocouple (ELLAB, CTD 85; ±0.05°C).

Data Processing. Steady state ME gas concentrations were converted to partial pressures, corrected to barometric pressure of 760 mm Hg of water vapor saturation pressure for the corresponding ME temperature (see Table). The ME ventilation-perfusion ratio ($\dot{V}$ME/$\dot{Q}$ME) was calculated according to Farhi,[18] assuming complete equilibration between the ME and venous blood gas pressures leaving the ME[19] (see Appendix[20,21]). Data are presented as mean ± SD. The influence of ventilation changes on blood and ME variables was tested by means of a logistic regression procedure (SPSS Statistical Software). Differences between treatments were tested with a paired *t*-test.

RESULTS

Spontaneous Breathing. During the steady state of spontaneous breathing, the arterial PO2 (PaO2) was 57.5 mm Hg, the venous PO2 (PvO2) was 33.2 mm Hg, and the ME PO2 was 36.8 mm Hg. The corresponding arterial PCO2 (PaCO2) was 46.0 mm Hg, the venous PCO2 (PvCO2) was 53.0 mm Hg, and the ME PCO2 was 70.4 mm Hg. These results were used as reference values (see Table).

Hyperventilation. Successful hyperventilation is evident from the significant decrease in PaCO2, the arterial pH increase, and the arterial bicarbonate ion decrease (see Table). The PaCO2 decreased from 46.0 mm Hg during spontaneous breathing to 17.9 mm Hg at the end of 30 minutes of hyperventilation ($p < .00001$). The PvCO2 decreased as well, from 53.0 mm Hg during spontaneous breathing to 37.5 mm Hg in hyperventilation ($p < .0072$; see Table). Similar changes in ME gas composition were simultaneously observed: the PCO2 decreased significantly, from 70.4 mm Hg during spontaneous breathing to 58.8 mm Hg during hyperventilation ($p < .04$).

No significant change in either PaO2 or PvO2 was recorded during hyperventilation as compared with spontaneous breathing ($p > .5000$ and $p > .2978$, respectively). Nevertheless, the ME PO2 decreased from 36.8 mm Hg to 27.3 mm Hg ($p < .0283$). During hyperventilation, as well as during spontaneous breathing, the ME PO2 was not significantly different from the PvO2, but the ME PCO2 was significantly higher than the PvCO2 ($p < .00001$).

Hypoventilation. Hypoventilation is evident from the decrease in PaO2, the increase in PaCO2, and the decrease in arterial O2 saturation (see Table). The PaO2 decreased from 57.5 mm Hg during spontaneous breathing to 48.3 mm Hg during hypoventilation ($p < .0931$), and the PvO2 decreased from 33.2 to 21.8 mm Hg ($p < .0126$), while the PaCO2 increased from 46.0 mm Hg during spontaneous breathing to 56.8 mm Hg during hypoventilation ($p < .0352$; see Table). In the ME, the PO2 decreased from 36.8 mm Hg to 25.4 mm Hg ($p < .0243$) and the PCO2 increased from 70.4 mm Hg to 88.8 mm Hg ($p < .0300$; see Table).

Switching from spontaneous breathing to either hyperventilation or hypoventilation led to a significant change in PCO2 in both arterial and venous blood. These changes resulted in a statistically significant correlation of ME PCO2 with both arterial and venous PCO2 ($r = .70$, $p < .0070$, and $r = .76$, $p < .0360$, respectively). In the PO2 there was a significant correlation between ME, arterial, and venous blood only for spontaneous breathing and hypoventilation ($r = -.86$, $p < .013$).

DISCUSSION

Middle ear gas composition is influenced by several factors: 1) its ventilation, eg, the composition, volume, and frequency of gas entering from the nasopharynx via the eustachian tube, 2) the diffusion capacity of the mucosal layer composing the walls of the ME for exchanging gases between the ME space and blood, 3) the O_2 consumption and CO_2 production rates of the mucosal tissue, 4) the blood gas composition, and 5) the rate of mucosal blood perfusion. The ME is considered to be a poorly ventilated gas pocket[18,20] — a fact that predicts an almost full equilibrium, during steady state, for O_2 and CO_2 partial pressures in the ME and the venous blood leaving it.[20,22] This has been demonstrated in guinea pigs when simultaneous measurements of these gases in ME and vein revealed ME gas pressure values that were more similar to vein values than to arterial ones.[7] The present study shows that as a result of pulmonary ventilatory changes, the PCO_2 and PO_2 in the ME follow those in the venous system. The mean ME PO_2 during spontaneous breathing (36.8 mm Hg), hyperventilation (27.3 mm Hg), and hypoventilation (25.4 mm Hg) is very close to the mean jugular PvO_2 (33.2, 29.5, and 21.8 mm Hg, respectively), while arterial blood values are very different (see Table and Fig 2).

The intriguing result is that the ME PCO_2 is consistently higher ($p < .00001$) than the $PvCO_2$, during spontaneous breathing steady state as well as during pulmonary ventilation changes: 70.4 versus 53.0 mm Hg in spontaneous breathing, 58.8 versus 37.5 mm Hg in hyperventilation, and 88.8 versus 66.4 mm Hg in hypoventilation (see Table and Fig 2). High ME PCO_2 values have been consistently measured by other groups as well: Ingelstedt et al[9] measured an ME PCO_2 of 58 mm Hg in humans, Segal et al[10] measured 53 mm Hg in guinea pigs, and Hergils and Magnuson[11] found 52 mm Hg in humans. However, Felding et al[12] and Grøntved et al[13] have found measured PCO_2 values in the ME to be closer to those in mixed venous blood: 48 and 50 mm Hg, respectively. The higher than venous blood PCO_2 recorded in ME at all pulmonary ventilation levels may be due to the site of venous blood sampling. We measured venous blood gases in the external jugular vein, which drains not only the ME, but all peripheral areas of the face, while the actual gas exchange in question takes place in the small venules around the ME only. Near equilibrium, a steady state is probably achieved between the ME gas pressures and these venules, across the mucosal tissue. Had blood been retrieved directly from the ME circulation itself, its PCO_2 values might have been more compatible with the ME gas ambience. If complete equilibrium exists between ME gas composition and the venous side of the ME circulation, one would expect not only a high ME PCO_2, but also a low ME PO_2 compared with the jugular vein values. However, the ME PO_2 is not significantly different from that measured in the external jugular vein. The higher than jugular vein blood ME PCO_2 values on one hand, and similar to venous blood ME PO_2 values on the other, may indicate that the ME mucosa offers a higher diffusion resistance to O_2 than to CO_2. This is in accordance with the much lower membranal solubility of O_2 compared with CO_2. A similar phenomenon was found in the air cell of the chick embryo, across the chorioallantoic membrane of the chick embryo.[23] Alternatively, metabolic pathways different from those for other facial areas drained by the external jugular vein must be assumed. Involvement of acid-base regulating mechanisms and the effects of anesthetics must also be considered for explaining the high ME PCO_2.[24]

Hyperventilation characteristically removes CO_2 from arterial blood. Indeed, the mean $PaCO_2$ decreased, from 46.0 to 17.9 mm Hg, while the $PvCO_2$ decreased to a lesser extent, from 53.0 to 37.5 mm Hg. This was accompanied by a similar decrease of ME PCO_2 from 70.4 to 58.8 mm Hg. An opposite trend was measured during hypoventilation: the $PaCO_2$ increased to 56.8 mm Hg, the $PvCO_2$ increased to 66.4 mm Hg, and the ME PCO_2 increased to 88.8 mm Hg (Fig 2). It is apparent that the ME PCO_2 follows the same trend of changes that occurred in blood during both hypoventilation and hyperventilation. The same holds true for O_2 as well. The mean ME PO_2 decreased from 36.8 to 25.4 mm Hg, following a PO_2 decrease in venous blood from 33.2 to 21.8 mm Hg, after the switch from spontaneous breathing to hypoventilation. These results indicate that ME gas composition changes follow dynamic blood gas composition changes.

The significant decrease in ME PO_2 during hyperventilation (see Table and Fig 2) contradicts our predictions. An increase rather than a decrease in ME PO_2 was expected. The fact that PaO_2 did not change significantly upon switching from spontaneous breathing to hyperventilation may indicate an ME local vasoconstriction induced by hyperventilation. To test this hypothesis, we have assumed, as a first approximation approach, a full equilibrium in N_2 partial pressure between the venous blood leaving the ME circulation and the ME cavity, and calculated the ME ventilation-perfusion ratio ($\dot{V}_{ME}/\dot{Q}_{ME}$; see Appendix for details). Our calculations indicate that $\dot{V}_{ME}/\dot{Q}_{ME}$ increased from 8.6×10^{-4} during spontaneous breathing to 12.9×10^{-4} in hyperventilation (see Table). Assuming that ME ventilation through the ET did not change (as may be the case in an anesthetized animal), an increase in $\dot{V}_{ME}/\dot{Q}_{ME}$ ratio means that blood perfusion decreased by 33%, denoting vasoconstriction. During hypoventilation, a reverse effect on $\dot{V}_{ME}/\dot{Q}_{ME}$ ratio was found: $\dot{V}_{ME}/\dot{Q}_{ME}$ decreased from 8.6×10^{-4} to 7.2×10^{-4}, indicating that a 19% increase in ME perfusion might have occurred. Such vasomotor reactions in the ME mucosa may be of significant importance for ME gas exchange, volume and pressure control, and acid-base regulation in the mucosa. A vasoactive influence of CO_2, either directly[25] or via the nitric oxide pathway, has been documented in the brain blood vessels, and the ME[26] may have a similar control. It seems that vasomotor regulation plays a role in determining the ME gas composition. Unfortunately, direct measurements of vasomotor changes and their influence on ME gas composition are missing and are very difficult to perform.

Since total ME pressure (the sum of individual gas partial pressures) is highly dependent on ME gas exchange, altering ME individual gas pressures by pulmonary ventilation changes, as demonstrated in the present work, will affect ME gas exchange and thus total ME pressure. The influence of hyperventilation on ME partial pressures and ME total pressure was indeed demonstrated by Buckingham et al[27] in dogs, when a total pressure of +20 mm H_2O at normal respiration decreased to −25 mm H_2O during hyperventilation. Our study indicates that this could have occurred mainly due to CO_2 depletion. Similarly, positive pressure is expected to develop during hypoventilation, due to an accumulation of CO_2 in the ME. It is possible that the higher morning pressures observed by Bylander et al,[28] Hergils and Magnuson,[29] and Luntz and Sadé[30] can be thus explained.

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APPENDIX

Calculation of middle ear ventilation-perfusion ratio ($\dot{V}\mathrm{ME}/\dot{Q}\mathrm{ME}$)

The $\dot{V}\mathrm{ME}/\dot{Q}\mathrm{ME}$ calculations are based on three assumptions. A) The venous blood leaving the ME mucosa is in equilibrium with the gas pressure in the ME space. B) Both N2 and O2 leave the ME cavity only as dissolved gases via the blood circulation; no gas leaves the ME in a gas phase, ie, through the eustachian tube. C) The arterial (a) N2 concentration is 79%,[20] and the N2 solubility coefficient at 37°C in blood is $\alpha\mathrm{N2} = 0.0127$ mL × (mL × 760 mm Hg)$^{-1}$. On the basis of these assumptions, the volume of N2 leaving the ME space per time unit (=$\dot{V}\mathrm{N2}$) at steady state is equal to the amount carried away by blood. This is the product of the ME blood flow ($\dot{Q}\mathrm{ME}$) times the amount of N2 carried away by each unit of blood flow:

(1) $\dot{V}\mathrm{N2} = \dot{Q}\mathrm{ME}\,(\mathrm{FMEN2} - \mathrm{FaN2})$

where F = volume fraction. Using assumption A and αN2, the nitrogen fraction can be transformed into partial pressure thus:

(2) $\dot{V}\mathrm{N2} = \dot{Q}\mathrm{ME} \times (\alpha/760) \times (\mathrm{PMEN2} - \mathrm{PaN2}) = \dot{Q}\mathrm{ME} \times (\alpha/760) \times (\mathrm{P_B} - \mathrm{PH2O}) \times (\mathrm{FMEN2} - \mathrm{FaN2})$

where P is partial pressure in mm Hg, $\mathrm{P_B}$ is the barometric pressure, and PH2O is the water vapor saturation partial pressure at measured temperature = 47 mm Hg at 37°C. The ME ventilation ($\dot{V}\mathrm{ME}$; gas entering through the eustachian tube) is given by

(3) $\dot{V}\mathrm{N2} = \dot{V}\mathrm{ME} \times \mathrm{FIN2}$

where $\dot{V}\mathrm{ME}$ is the average volume of gas entering the ME per unit of time from the nasopharynx and FIN2 is the N2 fraction in it (=0.79).[21] Combining equations 2 and 3 and assumption C results in

(4) $\dot{V}\mathrm{ME}/\dot{Q}\mathrm{ME} = 0.0119 \times (\mathrm{FMEN2} - \mathrm{FaN2})/\mathrm{FIN2} = 0.0151 \times (0.21 - \mathrm{FMEO2} - \mathrm{FMECO2})$.

FMEO2 and FMECO2 can be calculated from the data presented in the Table as ME PO2/(760 – 47) or ME PCO2/(760 – 47).

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MOLECULAR ANALYSIS OF THE POU3F4 GENE IN PATIENTS WITH CLINICAL AND RADIOGRAPHIC EVIDENCE OF X-LINKED MIXED DEAFNESS WITH PERILYMPHATIC GUSHER

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The molecular defect in some patients with X-linked mixed deafness with perilymphatic gusher at stapes surgery (DFN3) was recently attributed to mutations in the POU3F4 gene. In this manuscript we describe the molecular analysis of the POU3F4 gene in 5 patients with clinical and radiographic evidence of DFN3. Novel mutations were found in 2 of the 5 patients analyzed, while 3 had an entirely normal protein coding sequence. The fact that 3 of the 5 patients with clinical histories and radiographic abnormalities characteristic of X-linked mixed deafness with perilymphatic gusher displayed normal POU3F4 gene sequences supports the possibility that not all patients with the characteristic phenotype have involvement of the POU3F4 gene.

KEY WORDS — genetics, molecular analysis, perilymphatic gusher, X-linked mixed deafness.

INTRODUCTION

Approximately 0.1% of newborn infants have a hearing impairment, and 50% of these cases have a hereditary basis.[1] The vast majority of hereditary hearing loss is autosomal recessive. Fewer than 2% of all inherited losses are attributable to gene abnormalities on the X chromosome.[2] Five types of X-linked nonsyndromic deafness have been recognized (see Table[2]). X-linked progressive mixed deafness with perilymphatic gusher (DFN3) is the most frequently encountered of these, comprising approximately 0.8% of all cases of inherited deafness.[3] The other types of X-linked nonsyndromic deafness are summarized in the Table.

Olson and Lehman,[3] in 1968, reported on two maternal half brothers with early childhood mixed deafness, who, upon attempted stapedectomy, demonstrated perilymphatic gushers. They concluded that these cases represented a failure of differentiation of the stapedial annular ligament and attributed the perilymphatic gusher to a patent cochlear aqueduct. Nance et al,[4] in 1971, were the first to describe the X-linked pattern of inheritance of congenital mixed deafness with a stapes gusher.

Since these original descriptions, DFN3 has been well characterized.[5-10] The hearing loss consists of an early childhood bilateral progressive mixed loss affecting all frequencies.[8] Despite the conductive component to the loss, these patients often demonstrate brisk acoustic reflexes that contradict the theory of congenital stapes fixation.[8,10] Many affected individuals display reduction of vestibular function on electronystagmography.[7,8] A bilateral mixed or purely

FORMS OF X-LINKED NONSYNDROMIC DEAFNESS

Type	*Location*	*Clinical Features*
Congenital sensorineural loss	Xq13-q21	70- to 100-dB loss across all frequencies
Early-onset sensorineural loss	Xq21	Onset at age 3 to 4 years; possibly syndromic*
Moderate sensorineural loss	Unknown	Onset at age 10 years; progressive high-tone loss
High-frequency sensorineural loss	Unknown	Nonprogressive high-tone loss
DFN3	Xq21.1	See text

Table modified from Gorlin.[2]

*From Mohr J, Mageroy K. Sex-linked deafness of a possibly new type. Acta Genet (Basel) 1960;10:54-62.

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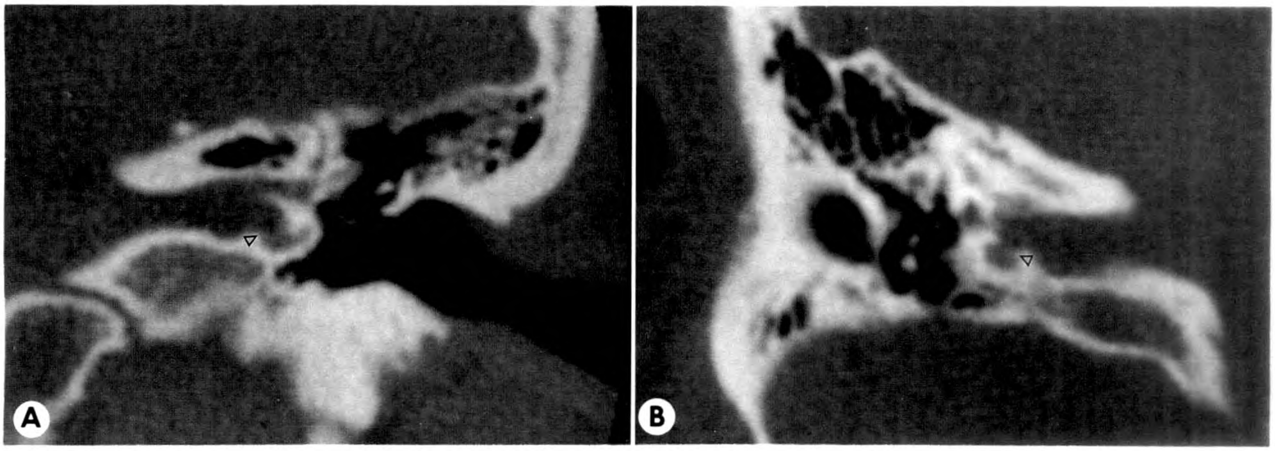

Fig 1. Computed tomograms demonstrating characteristic findings in DFN3 mutants. Arrowhead — absence of cochlear modiolus in fundus of internal auditory canal. A) Coronal. B) Axial.

sensorineural loss has been demonstrated in several female carriers.[5]

Glasscock[10] was the first to report the radiographic abnormalities in patients affected with this disorder. He described an abnormal dilatation of the lateral extent of the internal auditory canal (IAC) and a dilated vestibule. Cremers et al[7] corroborated the tomographic findings of Glasscock and also noted dilatation of the fallopian canal, small cochleas, and reduced diameter of the semicircular canals. With the advent of high-resolution computed tomography (CT), several authors have identified abnormalities of the cochlear modiolus, especially at the base, leading to direct communication between the subarachnoid and perilymphatic spaces (Fig 1).[11-13] The pathogenesis of the hearing loss, both the conductive and sensorineural components, and the rush of perilymph after stapedectomy is felt to be due to this widely patent communication.[5,10-13]

Recently, molecular defects in the POU3F4 gene were identified in 5 of 14 unrelated patients with X-linked deafness.[14] Six of the 14 patients had bony defects and/or a perilymphatic gusher, and of these patients, 4 had mutations.[14] The mutations were in the POU-specific domains and the POU homeodomains of the POU3F4 gene, with three of the mutations being small frameshift deletions that resulted in premature translational stop codons and two being missense mutations. The same group published a report on two additional families with DFN3, one of which had a frameshift deletion, and the other of which had a missense mutation.[15] In this report we describe the molecular analysis of 5 patients with clinical and radiographic findings characteristic of DFN3.

CASE REPORTS

Case 1. The clinical characteristics of this case have been previously described.[11] This patient has two brothers, one displaying a similar phenotype, and one suffering from blindness and cognitive delay. There is no other history of hearing loss in maternal relatives.

Case 2. The clinical characteristics of this case have been previously described.[11] This patient is one of two boys. His younger sibling displays a moderate bilateral sensorineural hearing loss. There are no maternal relatives known to have hearing loss.

Case 3. The clinical characteristics of this case have been previously described.[11] This patient is the only male child. His mother suffers from sensorineural hearing loss that began at the age of 54. The maternal grandmother suffered a hearing loss similar to that of the mother. A maternal granduncle and a male cousin displayed early childhood mixed deafness.

Case 4. This patient presented with a long history of bilateral moderately severe mixed hearing loss. At the age of 8 years he underwent stapedectomy on the left ear, at which time a profuse perilymphatic gusher was noted and reported to fill the ear and run out of the ear canal. Postoperatively the hearing loss was unchanged, and the patient has demonstrated a gradually progressive sensorineural loss over the following 27 years. High-resolution CT scan reveals bulbous and foreshortened IACs, widening of the labyrinthine portion of the fallopian canal, and an abnormal appearance of the basal portion of the modiolus. This patient is one of two boys. The younger brother was found to have bilaterally symmetric sensorineural hearing loss at a young age. There is no further information on the progression of his loss, and there is no history of hearing loss in any of the maternal relatives.

Case 5. The clinical characteristics of this case have been previously described.[13] On further review

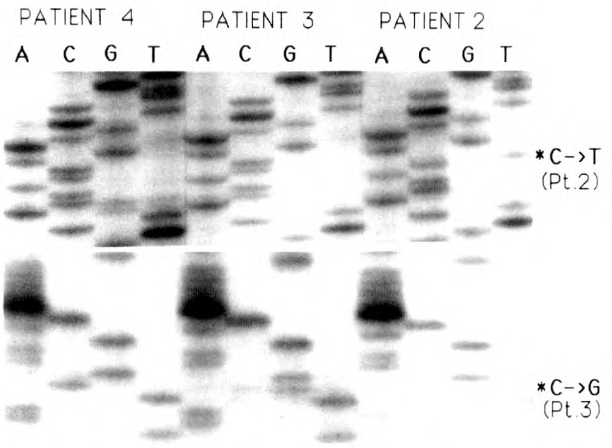

Fig 2. Autoradiographs of sequencing gels demonstrating mutations in patients 2 and 3. Upper panel shows sequences around mutation in patient 2 obtained with 5' primer, while lower panel shows sequences around mutation in patient 3 obtained with 3' primer. A, C, G, and T — four bases adenine, cytosine, guanine, and thymine.

of the patient's family history, three maternal uncles proved to have hearing loss dating back to early childhood, and two maternal granduncles had a history of long-standing hearing loss. No members of the family underwent stapes surgery.

MATERIALS AND METHODS

DNA Preparation. DNA was isolated from blood samples by the method of Old.[16]

Polymerase Chain Reaction. DNA was amplified[17] with 500 ng genomic DNA, 4 pmol of each primer (primer pairs as defined in de Kok et al[14]), 10 mmol/L Tris–hydrochloric acid, pH 8.0, 50 mmol/L potassium chloride, 1.5 mmol/L magnesium chloride, 200 µmol/L deoxynucleotide triphosphate, and 0.5 UTaq DNA polymerase (Gibco BRL, Life Technologies, Gaithersburg, Md) in a volume of 30 µL in a Perkin-Elmer/Cetus thermal cycler (Foster City, Calif). Cycling conditions included an initial 5-minute denaturation at 95°C, followed by 30 step-cycles of 94°C, 1 minute; 58°C (primer pair V) or 61°C (primer pair II) or 64°C (primer pairs I, II, IV), 1 minute; and 72°C, 1 minute, with a final elongation step for 5 minutes at 72°C.

Polymerase Chain Reaction Sequencing. Polymerase chain reaction products were directly sequenced with the Gibco BRL cycle sequencing system with deoxyadenine triphosphate labeled with γ-phosphorus 32 (Dupont, Boston, Mass). The sequencing products were electrophoretically separated on 6% polyacrylamide gel for 1 hour 15 minutes and 2 hours 30 minutes at 60 W. After vacuum drying, the gels were exposed to autoradiography film (Amersham, Arlington Heights, Ill) for 10 hours at –70°C.

RESULTS

To search for mutations in the POU3F4 gene, we sequenced the entire protein coding region of the gene from 5 unrelated patients with X-linked deafness.

We found that 2 patients had missense mutations in the POU3F4 gene (Figs 2 and 3[14,15]). In patient 2 there is a C to T transition at nucleotide 689, which results in an amino acid substitution from threonine to isoleucine at amino acid 230 (Thr230Ile) in the POU3F4 protein. This change occurs in the POU-specific domain, in which mutations have previously been described in 2 patients. The POU-specific domain binds to DNA at the ATGC sequence, which represents the 5' end of the octamer recognition sequence. The threonine residue at position 230 is located in helix 3 of the POU-specific domain, as

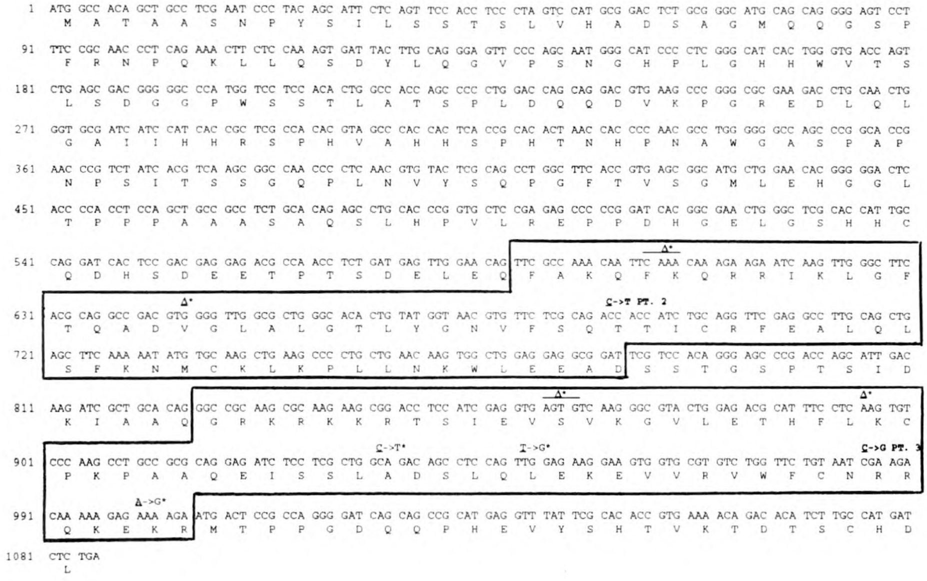
1 ATG GCC ACA GCT GCC TCG AAT CCC TAC AGC ATT CTC AGT TCC ACC TCC CTA GTC CAT GCG GAC TCT GCG GGC ATG CAG CAG GGG AGT CCT
M A T A A S N P Y S I L S S T S L V H A D S A G M Q Q G S P
91 TTC CGC AAC CCT CAG AAA CTT CTC CAA AGT GAT TAC TTG CAG GGA GTT CCC AGC AAT GGG CAT CCC CTC GGG CAT CAC TGG GTG ACC AGT
F R N P Q K L L Q S D Y L Q G V P S N G H P L G H H W V T S
181 CTG AGC GAC GGG GGC CCA TGG TCC TCC ACA CTG GCC ACC AGC CCC CTG GAC CAG CAG GAC GTG AAG CCC GGG CGC GAA GAC CTG CAA CTG
L S D G G P W S S T L A T S P L D Q Q D V K P G R E D L Q L
271 GGT GCG ATC ATC CAT CAC CGC TCG CCA CAC GTA GCC CAC CAC TCA CCG CAC ACT AAC CAC CCC AAC GCC TGG GGG GCC AGC CCG GCA CCG
G A I I H H R S P H V A H H S P H T N H P N A W G A S P A P
361 AAC CCG TCT ATC ACG TCA AGC GGC CAA CCC CTC AAC GTG TAC TCG CAG CCT GGC TTC ACC GTG AGC GGC ATG CTG GAA CAC GGG GGA CTC
N P S I T S S G Q P L N V Y S Q P G F T V S G M L E H G G L
451 ACC CCA CCT CCA GCT GCC GCC TCT GCA CAG AGC CTG CAC CCG GTG CTC CGA GAG CCC CCG GAT CAC GGC GAA CTG GGC TCG CAC CAT TGC
T P P P A A A S A Q S L H P V L R E P P D H G E L G S H H C
Δ*
541 CAG GAT CAC TCC GAC GAG GAG ACG CCA ACC TCT GAT GAG TTG GAA CAG TTC GCC AAA CAA TTC AAA CAA AGA AGA ATC AAG TTG GGC TTC
Q D H S D E E T P T S D E L E Q F A K Q F K Q R R I K L G F
Δ*
C->T PT. 2
631 ACG CAG GCC GAC GTG GGG TTG GCG CTG GGC ACA CTG TAT GGT AAC GTG TTC TCG CAG ACC ACC ATC TGC AGG TTC GAG GCC TTG CAG CTG
T Q A D V G L A L G T L Y G N V F S Q T T I C R F E A L Q L
721 AGC TTC AAA AAT ATG TGC AAG CTG AAG CCC CTG CTG AAC AAG TGG CTG GAG GAG GCG GAT TCG TCC ACA GGG AGC CCG ACC AGC ATT GAC
S F K N M C K L K P L L N K W L E E A D S S T G S P T S I D
Δ*
Δ*
811 AAG ATC GCT GCA CAG GGC CGC AAG CGC AAG AAG CGG ACC TCC ATC GAG GTG AGT GTC AAG GGC GTA CTG GAG ACG CAT TTC CTC AAG TGT
K I A A Q G R K R K K R T S I E V S V K G V L E T H F L K C
C->T*
T->G*
C->G PT. 3
901 CCC AAG CCT GCC GCG CAG GAG ATC TCC TCG CTG GCA GAC AGC CTC CAG TTG GAG AAG GAA GTG GTG CGT GTC TGG TTC TGT AAT CGA AGA
P K P A A Q E I S S L A D S L Q L E K E V V R V W F C N R R
A->G*
991 CAA AAA GAG AAA AGA ATG ACT CCG CCA GGG GAT CAG CAG CCG CAT GAG GTT TAT TCG CAC ACC GTG AAA ACA GAC ACA TCT TGC CAT GAT
Q K E K R M T P P G D Q Q P H E V Y S H T V K T D T S C H D
1081 CTC TGA
L

Fig 3. Known mutations in POU3F4 gene. First three nucleotides code for start signal of protein coding part of gene, and last three nucleotides code for stop codon. Boxed areas represent POU-specific domain (first box) and POU homeodomain, respectively. Mutations seen in our patients are indicated in bold, and patient affected with mutation is indicated beside it. Other, previously described mutations and deletions (Δ) in this gene, and associated with DFN3, are shown.[14,15] Affected nucleotides are marked by bar.

deduced from nuclear magnetic resonance and crystallographic studies.[18] Helix 3 is positioned in the major groove and makes all of the base contacts with the ATGC site, with the main bonds made by six amino acids, one of which is the threonine at position 230.[18] The POU-specific domain of nearly all POU genes contains threonine at this position, with the exception of two proteins containing a serine at this position, which is presumed to make similar contacts.[18]

Patient 3 showed a C to G transition at nucleotide position 985. This mutation results in an amino acid substitution from arginine to glycine at amino acid 329 (Arg329Gly) in the POU homeodomain. The arginine residue at position 329 resides in helix 3 of the POU homeodomain, and is conserved in five of eight members of the non-POU domain proteins that belong to the homeodomain superfamily and in all POU domain proteins except Brn3, where the arginine residue is substituted by glutamine.[19,20] Helix 3 of the POU homeodomain makes main contacts with the AAAT subsite in the major groove in the proposed crystal structure of the engrailed homeodomain-DNA complex. The function of Arg329 in the binding has not been elucidated, but the two adjoining amino acids, Cys327 and Asn328, make critical contacts with the AAAT site.[18]

In both cases, the location of the mutations, their evolutionary conservation, and their role in DNA binding or close proximity to DNA binding amino acids suggest a causal relationship between the mutation and the deafness phenotype.

DISCUSSION

Based upon variations in clinical presentation, five types of X-linked nonsyndromic deafness have been identified: 1) congenital sensorineural, 2) early-onset sensorineural, 3) moderate sensorineural, 4) high-frequency sensorineural, and 5) mixed deafness associated with perilymphatic gusher at stapes surgery, called DFN3 (see Table). Two linkage studies have provided evidence for the presence of a locus for DFN3 in the region Xq13-q21.1.[21,22] In each report, the patients had mixed hearing loss, and at least one member of each kindred displayed a perilymphatic gusher during surgery. Additional evidence for a locus at Xq21 has been provided by physical mapping of cytogenetically identified deletions and duplications in patients with complex syndromes including deafness, choroideremia, and mental retardation.[23,24] One of these patients was reported to have mixed deafness and a perilymphatic gusher. However, the other deletion patients had severe sensorineural losses.

Yeast artificial chromosomes spanning the putative region were isolated and an 850 kilobase cosmid contig was constructed, which allowed the identification of two microdeletions and a duplication in 3 DFN3 patients and the assignment of the DFN3 gene to a 500 kilobase interval within Xq21.1.[14,25] Subsequently, 5 patients with evidence of the disorder were found to have abnormalities in the POU3F4 gene, which is located in this interval.[14]

The simultaneous discovery of the pituitary-specific transcription factor, Pit-1, the B-cell–specific transcription factor, Oct-2, the ubiquitous octamer binding protein, Oct-1, and the *Caenorhabditis elegans* unc-86 gene product led to the identification of a novel DNA binding motif referred to as the POU domain.[26,27] The POU region consists of two highly conserved domains. The POU homeodomain is represented by a highly conserved amino-terminal region of 60 amino acids, and the POU-specific domain consists of an even more highly conserved 76-78 amino acid region. Detailed functional and genetic analyses of several POU domain factors have proven their roles as developmental regulators critical for the determination of cellular phenotypes in the central nervous system.[27,28] Experimental evidence exists that supports the likely role played by the mouse homolog of POU3F4 in inner ear development (Allen F. Ryan, personal communication, 1995). In situ hybridization studies of POU3F4 expression in the mouse reveal messenger RNA (mRNA) in the mesenchymal tissue adjacent to the ventral pole of the otocyst at embryonic day 12 (e12). On e14, expression appears in the mesenchyme adjacent to the dorsal pole of the otocyst and the overall level of expression is increased. By e16, high levels of expression are detected in all tissues of the developing inner ear except the neural cells and neuroepithelium. At this time point, mRNA levels are particularly high in the cochlear modiolus, and very little expression is detected in the otic capsule. The expression in these regions remains high until 12 days after birth (p12) and then declines abruptly on p14. From p14 to adulthood, low to moderate expression is detected in the spiral ligament, the spiral limbus, and the suprastrial cells.

In this report we have described 5 patients with clinical and radiographic evidence of X-linked mixed deafness with a stapes gusher. All of the patients demonstrated early-onset mixed deafness, and 4 displayed a perilymphatic gusher at surgery. The fifth patient never underwent attempted surgical correction of the conductive hearing loss. Two of the 5 patients displayed abnormalities in the sequence of the POU3F4 gene at sites that are critical for the DNA binding function of the protein. These findings are consistent with those of de Kok et al,[14] who found abnormalities in the POU3F4 gene sequence in several, but not all, patients with clinical and radiographic evidence of the disease. Furthermore, each of the mutations identified in this study and the previous studies exists within the POU domain, sparing the remainder of the sequence outside this domain.[14,15]

The identification of patients with clinical and radiographic evidence of the disease but no identifiable abnormality in the POU3F4 gene raises two possibilities. One possibility is that the remaining cases have mutations in adjacent cis-acting regulatory or structural elements that prevent transcription from the POU3F4 gene. In order to evaluate this possibility, we have tried by polymerase chain reaction amplification of cDNA to screen for "promiscuous" expression in lymphoblastoid cell lines, with no success (Fischel-Ghodsian et al, unpublished results). The second possibility is that mutations in an as-yet-undiscovered gene in the same chromosomal region are responsible for the remainder of the cases. Supporting this idea are three Xq21 microdeletions and one duplication in patients with clinical evidence of DFN3 that did not involve the POU3F4 gene but were 15 kilobases to 400 kilobases 5' to the gene.[25]

In conclusion, we have described the molecular defects in 2 of 5 patients with clinical and radiographic evidence of DFN3. The presence of sequence abnormalities in the POU3F4 gene of these individuals is consistent with the work of de Kok et al[14] and subsequent work by Bitner-Glindzicz et al,[15] and implicates this gene in the disorder. Furthermore, the presence of characteristic radiographic abnormalities in all of our patients with POU3F4 abnormalities corroborates the findings of the above-mentioned studies and supports the use of high-resolution CT for the diagnosis of DFN3. The absence of molecular defects in 3 of our patients with characteristic clinical histories and radiographic findings supports the possibility that this phenotype may be the result of a mutation in an as-yet-unidentified gene.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

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REPAIR OF THYROID CARTILAGE DEFECT WITH BONE MORPHOGENETIC PROTEIN

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The object of this research was to investigate whether a thyroid cartilage defect can be repaired by newly formed bone induced by bovine bone morphogenetic protein (bBMP). Bilateral thyroid cartilage defects measuring 0.5 × 0.6 cm were created in 25 adult rabbits. Experimental defects were implanted with ceramic bone particles combined with bBMP, or in controls, without bBMP. The animals were painlessly killed at 2, 4, 8, 12, and 16 weeks after implantation. The tissue responses were analyzed by routine histologic examination. An increase in the amount of new bone was observed in the interval from 4 to 12 weeks, and the defects were completely filled with new bone at 16 weeks, in contrast to a minimal new cartilage formation at the defect edges in controls. The results indicate that cartilage defects can be repaired by inducing new bone formation from mesenchymal cells in both perichondrium and muscular fascia.

KEY WORDS — bone morphogenetic protein, cartilage defect, laryngotracheal reconstruction.

INTRODUCTION

A laryngotracheal defect is a serious and difficult problem, since it causes laryngotracheal stenosis. Reconstruction of these defects requires the reestablishment of the cartilaginous supporting structure of the larynx and trachea. Numerous techniques, including using cartilage, bone, muscle, and artificial materials, have been tried with variable success. The ideal method for replacing lost laryngotracheal cartilage would be to induce growth of host replacement cartilage that would bridge an entire defect by means of a cartilage-inducing implant. So far, the cartilage-inducing factor has not been extracted. However, bone morphogenetic protein (BMP), a bone-inducing protein, has been extracted and applied in both animal experiments and clinical treatment for many years,[1-3] which has brought hope not only for orthopedists but also for otolaryngologists.[4,5] In this study, bovine BMP (bBMP) bound to ceramic bovine bone particles was used to repair 0.5 × 0.6-cm full-thickness thyroid cartilage defects in rabbits. The purpose of this study was to evaluate whether the bBMP implant could induce new bone to fill the defects in the cartilage.

MATERIALS AND METHODS

The bBMP fraction was prepared from bovine cortical bone by chemical extraction methods and partial purification by differential precipitation as previously described in detail,[1] and bioassayed for the capacity to consistently induce bone formation in a muscle pouch of the mouse thigh, having had no previous contact with bone tissue. Ceramic heterogeneous bone (CHB) particles were prepared by heating bovine costal bone to 900°C and grinding it mechanically to a particle size of 1 to 2 mm^3. One milligram of BMP was mixed with 5 mg of CHB particles and 5 μL of water, and the composite was shaped into a 5-mm square. As controls, composites made of 5 mg of CHB and 2 μL of water without BMP were used. Twenty-five adult male and female New Zealand White rabbits weighing 3.4 to 3.6 kg were divided into five groups and anesthetized with 2.5% sodium pentobarbital 20 mg/kg. Under sterile conditions, an incision was made in the middle of the neck and the thyroid cartilage was exposed. A 0.5 × 0.6-cm full-thickness defect including perichondrium was made in the middle of the cartilage on each side. The composites containing BMP were placed into the experimental defects on the right, while the composites without BMP were placed into the control defects on the left. The animals were painlessly sacrificed 2, 4, 8, 12, and 16 weeks after the operation, 5 at a time. The larynx was fixed in formalin, decalcified in 10% ethylenediaminetetraacetic acid, sectioned 5 μm thick, stained with hematoxylin and eosin, and observed by light microscopy.

RESULTS

Two weeks after implantation, the defect was filled with large quantities of spindle-shaped, fibroblastlike cells. Newly formed chondrocyte differentiation and cartilage matrix were partially present, and the ceramic bone particles of the composite remained in the center.

Four weeks after implantation, new bone and car-

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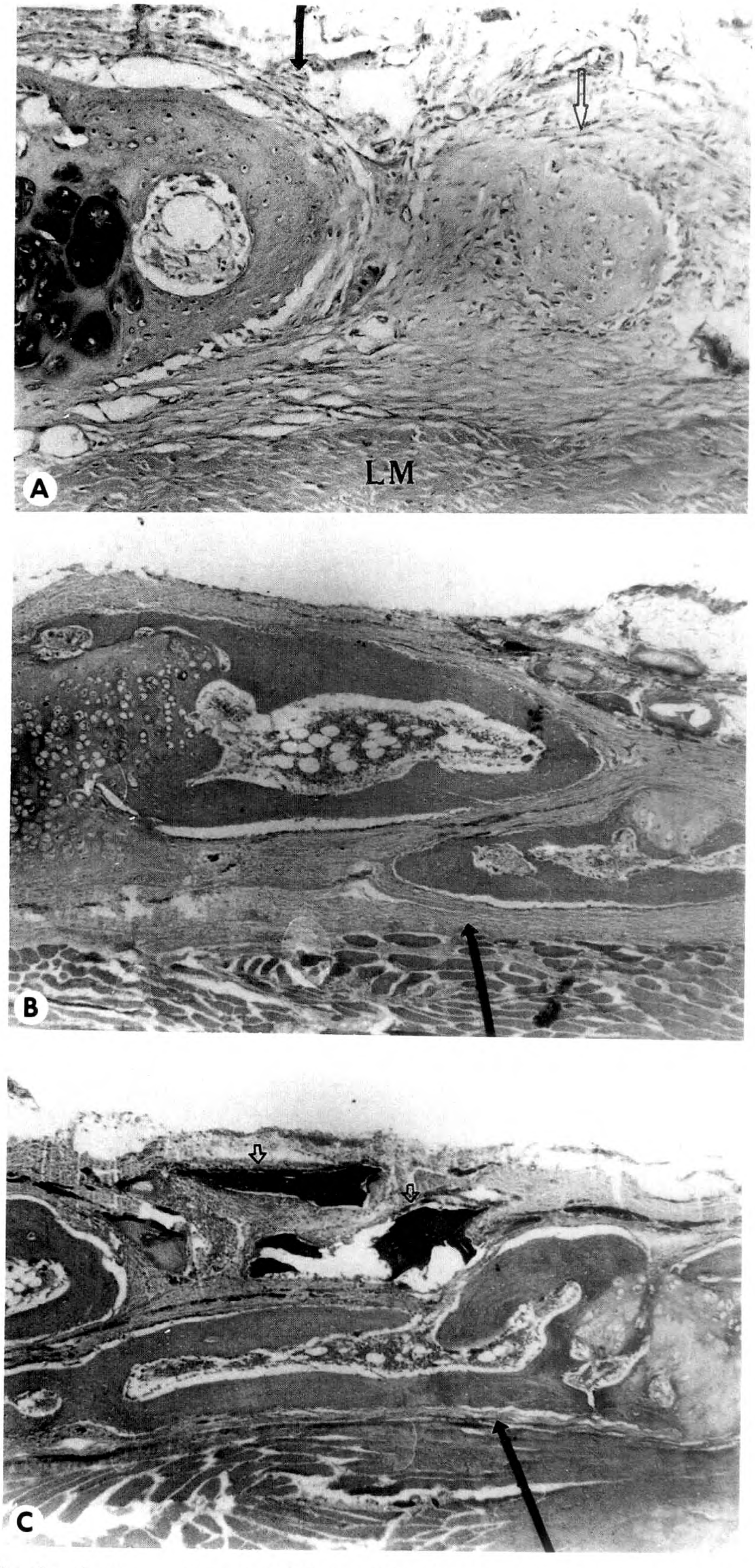

Results. A) Four weeks after implantation, showing new bone formation (closed arrow) at edge and new cartilage formation (open arrow) in middle of defect (original ×100). LM — laryngeal muscle. B) Eight weeks after implantation, showing enlargement of new bone and bone marrow (arrow; original ×40). C) Sixteen weeks after implantation, showing new bone formed completely across defect (closed arrow; original ×40). Note particles enclosed by fibrous connective tissues remaining along new bone (open arrows).

tilage formation had spread into the entire implant area. At the edge of the thyroid cartilage defect, newly formed trabecular bone and bone marrow were observed. Mesenchymal connective tissue, cartilage, and calcified cartilage were intermingled in the center of the implant (see Figure, A).

Eight weeks after implantation, the bone formation had advanced and woven bone was identified along the edges of the defect as well as in the centrally located islands of calcification. Woven bone often appeared to bridge the defect. The inside of the lamellar bone was filled with fatty bone marrow (see Figure, B).

The histologic organization of the defects in the 12-week group treated with BMP was similar to that described above for the 8-week experimental defects. There was extension of growth of woven bone and remodeled lamellar bone from the host cartilage defect rim.

In the 16-week BMP sample, there was evidence of complete closure of the defect. The thickness of the new bone was almost the same as that of the thyroid cartilage. Segments of unabsorbed ceramic bone particles were distributed along the newly formed bone (see Figure, C). No particles were found in the middle of new bone. In comparison, the control defect from the same time period showed only minimum newly formed cartilage at the edge of the defect.

DISCUSSION

Purified bBMP is an acidic protein with an isoelectric point of 5.0 ± 0.2, and a molecular weight of 18.5 kilodaltons.[1] It stimulates the irreversible induction and subsequent differentiation of mesenchymal cell components of the connective tissue into a population of osteoprogenitor cells,[6] resulting in the formation of supporting connective tissue, ie, cartilage and/or bone.

The possibility of inducing bone growth and regeneration has been evaluated by the implantation of BMP in experimental defects[2] and in patients with clinical bone defects.[3] In the present study, a 0.5 × 0.6-cm defect was completely closed by new bone that was almost as dense as the normal thyroid cartilage at 16 weeks. Microscopically, the cytologic and histologic reactions are the same in the defect of cartilage as in bones. A good fusion between the cartilage defect and new bone indicates a possible tight junction between them. In control groups, fibrous scar tissues bridge the defect, with minimal evidence of newly formed cartilage at the edge of the defect.

The target cell for BMP is the mesenchymal cell.[1] In the created defect in the thyroid cartilage, there are two kinds of tissues containing mesenchymal cells. One is the perichondrium at the edge of the thyroid cartilage defect, and the other is muscular fascia on the intralaryngeal and extralaryngeal muscles. It was noted that bone regeneration may occur not only from the thyroid cartilage rim, but also from the underlying muscular fascia. Therefore, maintenance of the integrity of perichondrium and fascia seems to be essential for complete regeneration beneath the cartilage margins and across the defect.

Soluble BMP requires a less soluble carrier for expression of its biologic potency in vivo. We believe that the ceramic bone particles used in this experiment are not a supporting material, but are a delivery system for BMP that prevents rapid outward diffusion and loss of the BMP molecules. Thus, in the presence of the particles, the content of BMP in implants and its concentration at interfaces between implants and the host remains high, enabling responding cells to come into contact with BMP molecules long enough to initiate bone induction.[7] Urist et al[8] implanted a composite of β–tricalcium phosphate and BMP into the thigh muscle pouch of mice and found the field of new bone was more than 12 times greater than that with BMP alone. Although the ceramic bone particles are not absorbed by the host and are finally enclosed by dense fibrous connective tissues, they do not seem to disturb the continuity and intactness of the new bone (see Figure, C).

ACKNOWLEDGMENTS — We thank Lian-Jia Yang, MD, and Yu-Hao Gao, MD, Department of Oral Pathology, College of Stomatology, Fourth Military Medical University, Xi'an, for their technical assistance in preparing the bBMP.

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CHEMOPROPHYLAXIS OF FUNGAL DETERIORATION OF THE PROVOX SILICONE TRACHEOESOPHAGEAL PROSTHESIS IN POSTLARYNGECTOMY PATIENTS

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A double-blind randomized trial was conducted among 36 laryngectomees to assess the influence of a buccal bioadhesive slow-release tablet containing miconazole nitrate on the lifetime of the Provox voice prosthesis. All patients colonized with *Candida* spp and treated with miconazole showed a significant decrease of colonization at the end of the study. Intratracheal phonatory pressures were remarkably higher after 2 months of follow-up in the placebo group. No local or systemic adverse reactions to miconazole were observed during this study. Patient compliance was acceptable according to regular miconazole determination in saliva samples. The device lifetime was significantly higher in patients treated with miconazole even after 1 year of follow-up. The use of a buccal bioadhesive slow-release tablet containing an antimycotic agent proves to be an adequate method of preventing fungal colonization and deterioration of silicone voice prostheses.

KEY WORDS — bioadhesive tablet, chemoprophylaxis, fungal deterioration, miconazole nitrate, total laryngectomy, voice prosthesis.

INTRODUCTION

Restoration of speech after total laryngectomy is generally considered to be a primary task in the rehabilitation of the laryngectomee. Since the first laryngectomies performed by Watson (1866) and Billroth (1873), several methods to restore speech have been attempted.[1,2] Alaryngeal speech production requires the establishment of an alternative vibratory source in the reconstructed pharyngoesophageal region. Speech restoration can be achieved by injection and/or shunt esophageal techniques, or by means of an artificial larynx that as an electric pharyngeal speech vibrator can be externally applied to the neck. The method of shunt esophageal speech by means of silicone tracheoesophageal valve prostheses was further developed after the introduction of the Blom-Singer duck-bill prosthesis in 1979.[3] Compared to the other methods of voice rehabilitation, consistent high success rates of shunt esophageal speech were obtained with several types of tracheoesophageal voice prostheses.[4] Further research was directed toward the development of a device that could be easily applied after primary or secondary puncture, that enables short-term acquisition of functional alaryngeal speech, and that requires only simple maintenance for optimal performance. This resulted in the development of indwelling, biocompatible silicone prostheses with a low-resistance one-way valve mechanism.

Rational patient selection and additional surgical management of the pharyngoesophageal segment had already contributed to improvement of prosthetic voice rehabilitation. Device life is known to be limited due to dysfunction of the valve mechanism. Colonization and invasion of silicone material with *Candida* spp has been reported to correlate with valve dysfunction.[5,6] The latter may be caused by leakage of esophageal contents through the prosthesis into the trachea as a result of insufficient valve closure, or by an increased expiratory airflow resistance during phonation. This will gradually lead to increased efforts to produce fluent shunt esophageal speech. Selective oropharyngeal yeast decontamination may prevent early deterioration of valve prostheses.[7,8] Systemic and topical antimycotics are currently used to treat oropharyngeal fungal infections.[9] Successful decontamination of the oropharyngeal yeast flora has been established with several antifungal drugs in immunocompromised and intensive care patients.[10,11] Topical agents (ie, lozenges and semisolid formulations) might adequately resolve superficial fungal infections, but must be applied frequently to maintain

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effective saliva concentrations.[12] A rapid decrease below the minimal inhibitory concentration is usually noticed after local application of these formulations. Application of a bioadhesive slow-release tablet has proven to maintain effective salivary drug concentrations for 10 to 12 hours after application on the buccal mucosa.[12] This could practically diminish the need for frequent drug applications with lower daily dosages. Fewer daily applications might improve patient compliance, while less drug is needed for long-term prevention of deterioration of silicone voice prostheses. A double-blind randomized study was conducted to assess the efficacy of bioadhesive slow-release tablets containing miconazole nitrate on the device life and function of the Provox tracheoesophageal voice prosthesis.

SUBJECTS AND METHODS

Subjects. Thirty-six patients who underwent total laryngectomy (34 men, 2 women; ages varying from 41 to 87 years; mean 63.1 years) were selected to participate in this prospective study. In our department all laryngectomees who received a voice prosthesis were selected according to specific criteria as described in a previous report.[13] All but 2 patients had a primary prosthesis insertion at the time of laryngectomy. Both secondary insertions were performed after a period of approximately 7 months. All participants received radiotherapy before or after laryngectomy (60 to 70 Gy). According to criteria proposed at the Third International Congress on Voice Prostheses (Groningen, 1988), all participants were able to produce functional shunt esophageal speech for at least 3 months. The patients were selected to start participation in the trial at the time when replacement of their voice prosthesis was required due to leakage or increased efforts to phonate properly. In all subjects a new Provox indwelling, low-resistance voice prosthesis was inserted as a standard outpatient procedure. Patients were included in the study after appropriate explanation of the trial procedures and signature of an informed consent document. Participants were allowed to prematurely drop out on individual request. Patients known to have allergic or other adverse reactions to miconazole were excluded from participation. No other antimicrobial agents were used simultaneously.

Study Design. A prospective double-blind randomized placebo-controlled study was designed to determine the efficacy of a bioadhesive, slow-release tablet containing 10 mg of miconazole nitrate on fungal colonization and dysfunction of the valve mechanism of the Provox voice prosthesis. Trial randomization, as well as production and supply of the pharmaceutical agents, was directed by the Laboratory of Pharmaceutical Technology (University of Ghent, Belgium). Tablets containing miconazole and placebo were equally distributed among the 36 participants (18 versus 18). Patients were instructed to administer the bioadhesive tablets twice daily, alternating between both sides of the buccal mucosa. At the start of the study, culture samples were taken from the oropharynx (dorsum of tongue), the tracheoesophageal shunt, and the esophageal flange (valve part) of the prosthesis. Immediately following prosthesis replacement, intratracheal phonatory pressures were assessed at three voice intensity levels. The efficacy of shunt esophageal speech could readily be assessed by estimating the intratracheal air pressure during phonation.[14] The trial was conducted over 6 months. Participants were evaluated monthly at our outpatient department for intratracheal phonatory pressure measurements, prosthesis inspection, and microbiologic assessment. Culture swabs were taken from the oropharynx, prosthesis (valve part), and tracheoesophageal shunt. Swabs were taken with suitable cotton-tips and preserved in vials without any transport medium. Daily maintenance of the prosthesis was performed with specifically manufactured brushes without any antiseptic solution. High-performance liquid chromatography was used to assess the concentration of miconazole in saliva samples.[12] Samples were taken at regular intervals to determine patient compliance.

The trial was considered terminated after a follow-up of 6 months, or sooner in case prosthesis replacement was required. The participants, however, were allowed to continue the use of the tablets.

Any occurring adverse effect or complication was recorded. A questionnaire with possible side effects was included for the patients to complete at each visit. In order to assess long-term device life, all patients were regularly seen up to 1 year after participation. The study proceeded according to the Declaration of Helsinki. It was approved by the Committee for Medical Ethics of the Medical Faculty, University of Ghent, on July 5, 1993 (project 93/84).

Bioadhesive Slow-Release Tablets. After simple application on the buccal mucosa in the region of the right or left upper canine, the tablet is fixed for 1 minute by slight manual pressure on the lip. Sticking of the tablet to the lip is prevented by moistening the tablet with the tongue. The bioadhesive slow-release tablets are sufficiently attached for 10 to 12 hours. As described in a previous study, no dislodgment of the tablets occurred during meals.[12] Also, no complaints of a disturbing sensation or dysgeusia were mentioned.

The bioadhesive slow-release tablets (total mass

100 mg, 7 mm diameter, 2 mm thick) are compounded of starch, polyacrylic acid 5%, and 10 mg of miconazole nitrate.[12] Miconazole nitrate is a synthetic imidazole derivative that has broad-spectrum activity against almost all pathogenic fungi except *Aspergillus* spp and Zygomycetes. It is considered to be the drug of choice in the treatment of superficial fungal infections.[15] In the literature, side effects due to the use of miconazole after local application are reported to be variable.[9] In preliminary trials, no side effects were evident.[12] During therapy, resistance to this drug is rare. The mycelia are less susceptible than the parasitic forms of dimorphic fungi.[16] Its mode of action consists of ergosterol synthesis blockage in fungi, leading to the production of a defective fungal cell membrane with altered permeability.[15]

Intratracheal Pressure Measurements During Phonation. At the start of the study and at each monthly visit, the participants were requested to phonate at three intensity levels for at least 10 seconds. At low (30 to 40 dB), normal (50 to 60 dB), and high intensity levels (70 to 80 dB), the intratracheal pressure was recorded during phonation of a verb (/á/). Sound intensity measurements were performed by the Computerized Speech Lab (model 4300, Kay Elemetrics Corporation, Pine Brook, NJ), with a boom-mounted microphone used to standardize microphone-to-mouth distance (30 cm). A portable manometer with an electromagnetic pressure sensor (type EMA 150, range 0 to 300 mm Hg, Dimed SA Electronic Engineering, Antwerp, Belgium) was fitted in an adapted Shiley cuffed cannula, which enclosed the tracheostoma sufficiently with silicone adhesives. This method was not likely to interfere with shunt esophageal speech, since the voice prosthesis was located at 1 to 2 cm below the posterior tracheocutaneous border.

Mycological Assessment. All culture swabs were assessed on a selective medium in a qualitative and semiquantitative way. Half of each sample was inoculated on a yeast isolation plate with Sabouraud dextrose agar, and the other half on a plate with Sabouraud dextrose agar containing chloramphenicol. Following inoculation, the swab was spread out over one half of the isolation plate, and over the other half successively after rotation of 180°. Next, the culture swabs were put into brain-heart infusion broth. Both plates and broth were then incubated at 25°C for 48 hours. A semiquantitative scoring system was used to determine the growth densities on the isolation plates. In accordance with this scoring system, the results were represented by colonization indices:

(–) No growth or less than 100 colonies on one half of the plate.

(1+) Isolated colonies (200 to 250) on only one half of the plate.

(2+) Total overgrowth (>300) of colonies on the primary half of the plate without evidence of growth on the opposite half.

(3+) Total overgrowth (>300) on the primarily inoculated half of the plate and isolated colonies (200 to 250) on the opposite half.

(4+) Total overgrowth of the whole plate.

Isolated yeast colonies were identified by means of the following criteria[17]: germ-tube test, morphology on cornmeal–Tween 80 or rice agar, and sugar assimilation test. The identity of *Candida albicans* was confirmed by detecting the activity of specific enzymes (hexosaminidase, proline arylamidase) with two commercially available test kits[18,19]: Rapidec albicans (Bio Mérieux, Marcy l'Etoile, France) and Albicans Screen test (Carr Scarborough Microbiologicals, Stone Mountain, Ga). Secondary cultures were made on Albicans ID agar (Bio Mérieux) that contained a chromogenic hexosaminidase substrate.[20] In certain cases an API 20 C AUX test strip was used for confirmation.[21]

Evaluation of Deposits. At every monthly visit, the prostheses were examined with short rigid 0° and 25° endoscopes (Wolf SL, Lumina series). A semiquantitative scoring system was used to determine the contamination of the valve part of the prosthesis. Depending on the number of deposits that were macroscopically evident on the hinge valve, a semiquantitative evaluation of contamination was proposed: no deposits (0), a single deposit (1), two or three deposits (2), or more than three deposits (3).

Analysis of Data. All data are given as mean values (±SD). The mycological data, intratracheal pressure measurements, and deposits on the prostheses were statistically evaluated with nonparametric statistics.[22] The Wilcoxon-Mann-Whitney test and the Friedman two-way analysis of variance test have been used to compare both groups. A p value of less than .05 was considered statistically significant. The effect of time (months) on the colonization indices was determined with repeated measurements using MANOVA (SPSS, SPSS Inc).

RESULTS

At the start of the study, both groups (miconazole versus placebo) appeared to be comparable. All patients selected to participate had their prostheses replaced because of valve dysfunction. These devices had been used for a period ranging from 3.5 to 14 months (mean 5.2 months). All replacements were

TABLE 1. SURVIVAL OF VOICE PROSTHESES DURING FOLLOW-UP PERIOD OF SIX MONTHS

		Months					
	Start	*1*	*2*	*3*	*4*	*5*	*6*
Total	36	34	34	33	31	28	27
Placebo	18	16	16	15	13	11	10
Miconazole	18	18	18	18	18	17	17
Noncolonized*	16	14	14	14	14	12	12
Placebo	8	6	6	6	6	5	5
Miconazole	8	8	8	8	8	7	7
Colonized*	20	20	20	19	17	16	15
Placebo	10	10	10	9	7	6	5
Miconazole	10	10	10	10	10	10	10

*Colonization of tracheoesophageal fistula at start of study.

performed in our outpatient department. Only 2 participants in the placebo group dropped out of the study within the first month, because of shunt insufficiency with external leakage and tumor recurrence. Their results were excluded from further analysis. During the 6 months of follow-up, prosthesis replacement was necessary as a result of minor internal leakage in 7 patients (6 placebo versus 1 miconazole) after a mean period of 4.6 months (Table 1). One of the replaced prostheses that was used by a patient receiving miconazole did not show any accumulation of deposits or debris. Dysfunction was probably caused by excessive cleaning, which could have damaged the hinge valve of the prosthesis. Evaluation 1 year after the start of this study revealed a substantially extended mean device life in patients who were treated with miconazole compared to the placebo group (9.3 versus 5.6 months; Table 2). Prosthesis replacement was necessary only 7 times in the miconazole group (n = 18), while a prosthesis was replaced in the placebo group 18 times (n = 16). No complications concerning the prosthesis or tracheoesophageal fistula related to the use of the bioadhesive tablet were noticed. Two patients of the placebo group had a minor, self-limited infection of the tracheoesophageal shunt just after prosthesis replacement. Both patients were treated with silver nitrate because of the formation of granulation tissue at the edge of their fistula. The bioadhesive tablets were all well tolerated by the patients. In both the miconazole-treated group and the placebo-treated group, no local or systemic side effects were recorded. Also, no application difficulties were mentioned. All tablets became eroded. None of the patients suffered from local irritation. According to the regular determination of miconazole in saliva samples (n = 424), patient compliance (97.3%) was most satisfactory. In the group of patients treated with miconazole (n = 18), the mean drug level (47.58 ± 46.13 $\mu g \cdot mL^{-1}$) was higher than the minimal inhibitory concentration of miconazole against *Candida* spp (1.56 $\mu g \cdot mL^{-1}$). During the study the minimal inhibitory concentrations against several initially isolated *Candida* strains remained unchanged. This may indicate that development of resistance against miconazole did not occur.

TABLE 2. DEVICE LIFE AFTER ONE YEAR OF FOLLOW-UP

	Device Life (mo)	
	Range	*Mean*
Pretreatment period (n = 36)	3.5 - 14	5.2
Placebo group (n = 16)	3.2 - 9	5.6
Miconazole group (n = 18)	4.3 - 12	9.3

Mycological Findings. The inventory mycological findings of the oropharynx of all 36 participating subjects were similar. No significant differences in colonization index (p > .05; Wilcoxon-Mann-Whitney test) between the placebo and miconazole-treated groups were observed (Fig 1). Subjects in the miconazole-treated group started with a significantly higher colonization index (p < .05; Wilcoxon-Mann-Whitney test) at the site of the fistula compared to the placebo group. During the first 3 months of this study, no significant differences in colonization index (p > .05; Wilcoxon-Mann-Whitney test) were found between the groups at the site of the tracheoesophageal fistula and prosthesis. A significantly lower colonization index (p < .05; Wilcoxon-Mann-Whitney test) was found in the oropharynx in the miconazole-treated group during the entire study period. In this treatment group the colonization appeared to be significantly lower (p < .001; Wilcoxon-Mann-Whitney test) after 3 months at the site of the fistula and prosthesis.

Fourteen patients (6 treated with placebo and 8 treated with miconazole) were not colonized with yeast species at the site of the tracheoesophageal fistula at the start of the study. Six patients receiving miconazole remained free of any fungal infection at the valve part of the prosthesis, while 2 other patients also treated with miconazole nitrate showed a minor fungal infection (1+) that had diminished at 6 months

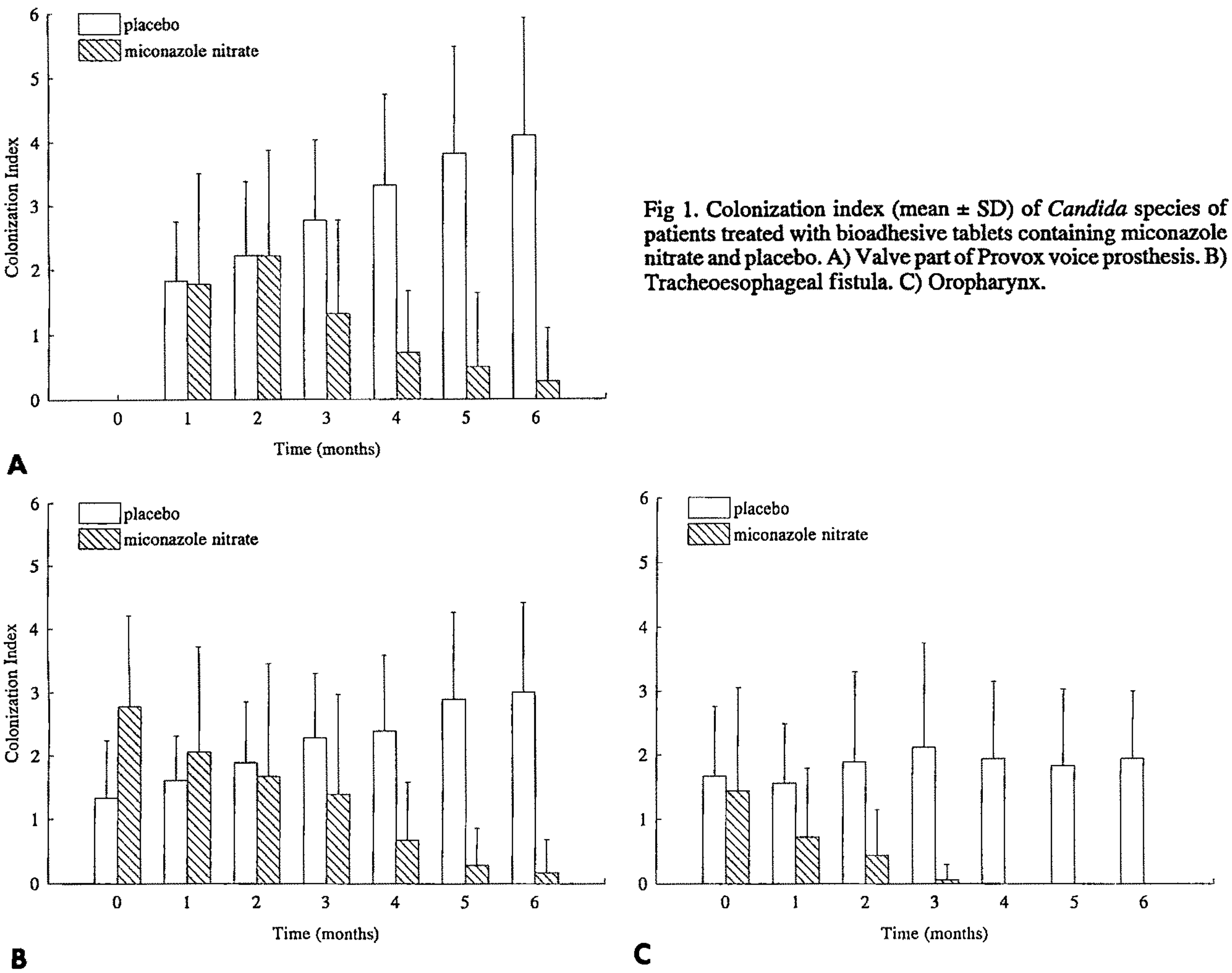

Fig 1. Colonization index (mean ± SD) of *Candida* species of patients treated with bioadhesive tablets containing miconazole nitrate and placebo. A) Valve part of Provox voice prosthesis. B) Tracheoesophageal fistula. C) Oropharynx.

of follow-up. In the placebo group, 5 out of the 6 patients who had negative inventory cultures showed a significant increase of the colonization index ($p < .001$; Friedman test) after 1 month of follow-up. One patient remained free of fungal infection.

In the miconazole-treated group (10 patients) with positive inventory cultures, all but 1 patient demonstrated a full suppression of fungal colonization at the valve part of the prosthesis (Fig 2). In the placebo group, all 10 patients remained with positive cul-

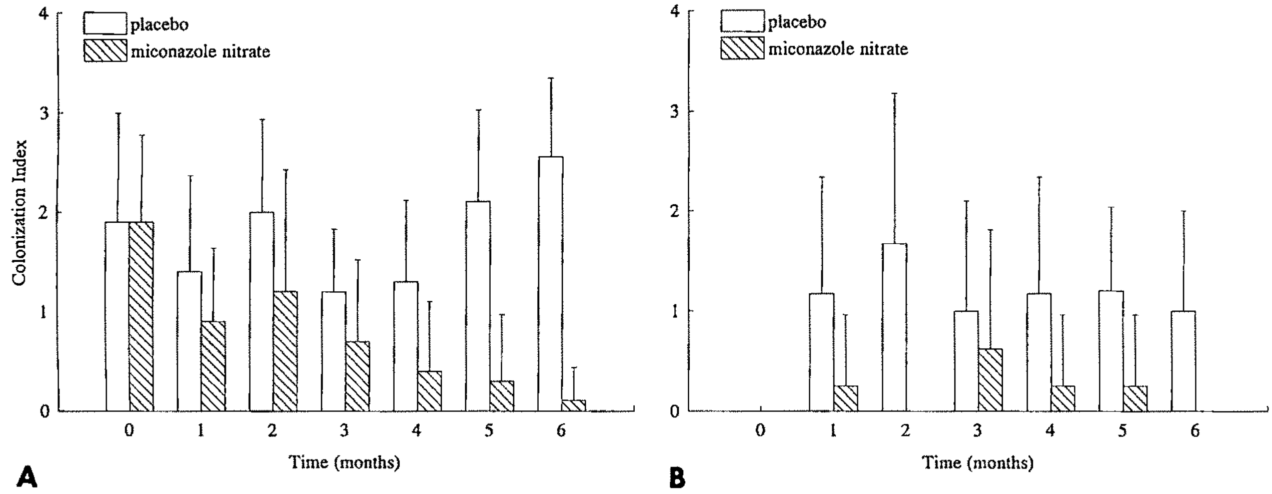

Fig 2. Colonization (mean ± SD) of valve part of Provox voice prostheses of patients treated with bioadhesive tablets containing miconazole nitrate and placebo, who had A) positive or B) negative inventory cultures.

TABLE 3. EVALUATION OF FUNGAL COLONIZATION OF VALVE PROSTHESIS

	Placebo *(n = 16)*		*Miconazole* *(n = 18)*	
	No.	*%*	*No.*	*%*
Negative cultures*/ all cultures†	22/104	21.2	86/125	68.8
Severe infections (3+)*/all cultures†	18/104	17.3	6/125	4.8
Negative cultures at 6 mo*/all cultures†	2/16	12.5	17/18	94.4
Severe infections (3+) at 6 mo*/all cultures†	4/16	25	0/18	0

*Of valve prosthesis.
†Of all three sites.

tures.

An obvious reduction of colonization ($F(6,300) = 32.08$; $p < .001$; MANOVA) at all three sites was found in the group of patients treated with a bioadhesive tablet containing miconazole. However, the decrease of colonization was significantly different ($F(12,294) = 1.80$; $p < .05$; MANOVA) at all three sites. In contrast, a remarkable increase of yeast colonization ($F(6,300) = 32.15$; $p < .001$; MANOVA) was noticed in the group of patients treated with placebo, except at the site of the oropharynx ($F(6,96) = 1.08$; $p = .381$; MANOVA). However, no clinical signs of an active *Candida* infection were found at the site of the fistula or oropharynx. In both the miconazole-treated group and the placebo group, the colonization indices were significantly different ($F(1,50) = 8.21$; $p < .001$; MANOVA) at all three sites during this study.

At 6 months of follow-up, 17 of 18 patients (94%) treated with miconazole had no fungal infection at the valve prosthesis (Table 3), while in the placebo group only 12.5% of the patients were free from any fungal infection. Twenty-five percent of the patients in the placebo group demonstrated a severe fungal infection (3+) at their valve prosthesis.

A high percentage (>80%) of patients appeared to be carriers of *Candida* spp. The following fungi were identified: *C albicans* (44%), *Candida glabrata* (25%), *Candida tropicalis* (14%), *Candida krusei* (7%), and, to a lesser degree, *Candida guilliermondi* and *Candida parapsilosis*. In a few cases *Candida famata* (*Torulopsis candida*), *Candida lusitaniae*, and *Candida pseudotropicalis*, together with other yeasts, were identified. Other organisms (ie, *Staphylococcus aureus*) were found in minor concentrations. Different *Candida* species were found to alter-

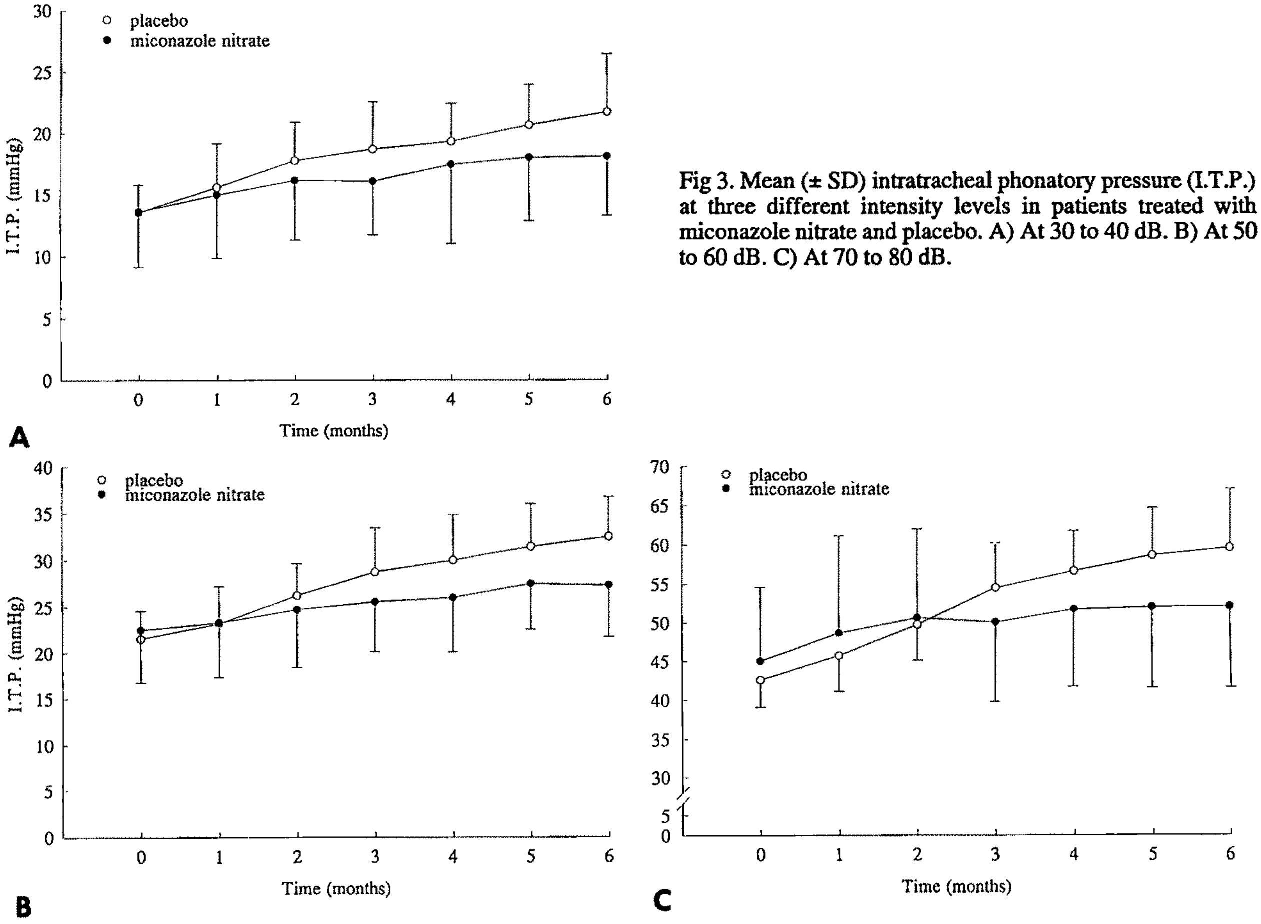

Fig 3. Mean (± SD) intratracheal phonatory pressure (I.T.P.) at three different intensity levels in patients treated with miconazole nitrate and placebo. A) At 30 to 40 dB. B) At 50 to 60 dB. C) At 70 to 80 dB.

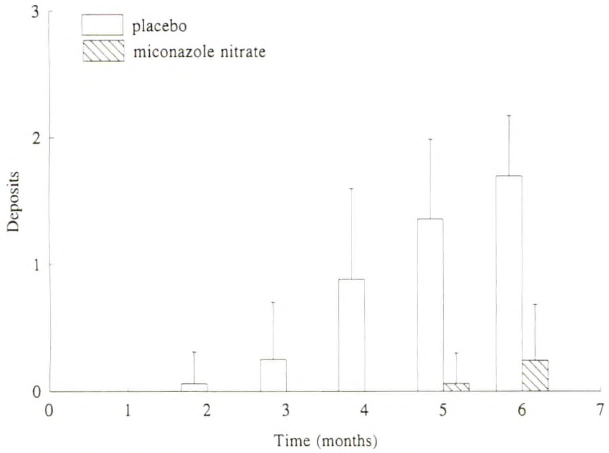

Fig 4. Evaluation of deposits (mean ± SD) on Provox voice prostheses in patients treated with miconazole nitrate and placebo.

nate in several executed cultures. These data are in accordance with other reports.[5,6,23]

Intratracheal Phonatory Measurements. The results of the intratracheal phonatory measurements at various intensity levels are displayed in Fig 3. In both the miconazole-treated and the placebo groups, a significant increase ($p < .001$; Friedman test) of the intratracheal pressures at all three intensity levels during the 6 months of follow-up was found. No significant differences in intratracheal phonatory pressures ($p > .05$; Wilcoxon-Mann-Whitney test) were noticed in both groups at the start, or 1 to 2 months after insertion of the Provox voice prostheses. After 3 months of follow-up, significantly lower intratracheal phonatory pressures were measured at all three intensities in the group of patients receiving miconazole, and the difference progressed during the following months.

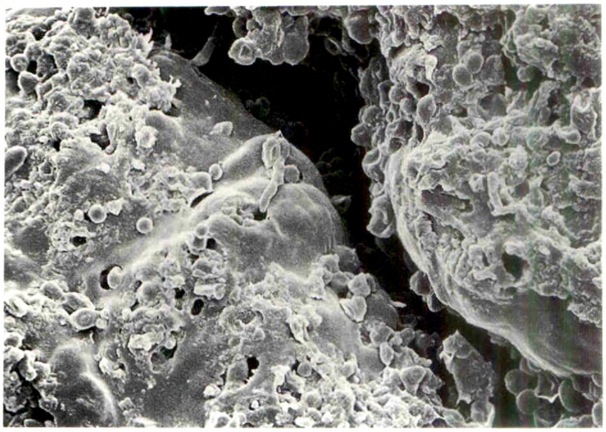

Fig 6. Electron microscopic details of fungal deterioration of silicone material of Provox voice prosthesis at esophageal end (scale, 1 cm = 5.4 μm).

Assessment of Deposits on Voice Prostheses. Only moderate deposits (1+) were macroscopically found on the voice prostheses of patients using bioadhesive tablets containing miconazole. One patient showed deposits (1+) on the valve part of the prosthesis after 5 months of insertion, while 3 other patients had similar deposits at the end of the study. After 4 months of insertion, a significant increase of deposits ($p < .001$; Friedman test) was demonstrated on the prostheses in patients treated with placebo (Fig 4). At the end of the study all patients receiving placebo, except 1, had moderate to severe deposits (1 to 3+) on their valve prostheses (Fig 5). The deposits were mainly located in the shaft, on the valve, and on the esophageal flange surrounding the hinged valve. Six of the valve prostheses that were replaced during this trial due to minor internal leakage showed heavy deposits and various debris around the esophageal flange. All deposits appeared to be firmly attached to the silicone material. Removal of the deposits was not possible without damaging the surface of the silicone

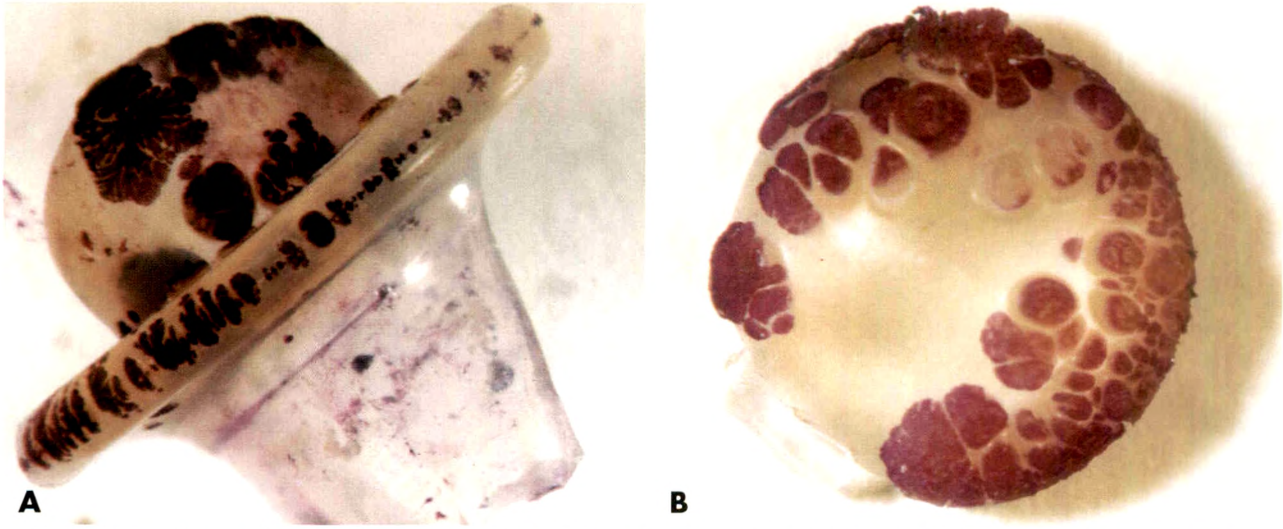

Fig 5. Stereomicroscopic views of A) esophageal end and B) valve part of Provox voice prosthesis with deposits (original ×12).

prosthesis. Scanning electron microscopic imaging of the esophageal part of dysfunctional prostheses showed both superficial defects and penetration of hyphae of *Candida* spp in the deeper layers of the silicone material, surrounded by other microbes (Fig 6).

DISCUSSION

Voice and speech rehabilitation after total laryngectomy can be successfully achieved by using several types of prosthetic devices. Application of indwelling, low-resistance voice prostheses has further improved the quality and fluency of shunt esophageal speech.[13] The main advantage of self-retaining devices might be the limited maintenance procedures, which could be responsible for the low complication rates as reported.[13,24] The device life may be influenced by regular use and maintenance, therapeutic irradiation, and colonization with yeast species. Deterioration of the silicone material will finally result in dysfunction of the one-way valve mechanism, leading to improper closure and increased airflow. The oral cavity and oropharynx are believed to be the continuous suppliers of yeast species and other microorganisms to the prosthesis located in the tracheoesophageal shunt.[5] Certain foods and beverages are also considered to be contributors of several fungi. The prevalence of yeast in the oral cavity is estimated to vary between 20% and 40% in healthy individuals.[25] Radiation to the head and neck regions, combined with surgical therapy, may further alter the oral flora and saliva composition to favor oral yeast colonization.[26] In studies with the standard low-resistance Groningen button, successful decontamination of the oropharynx with amphotericin B lozenges (10 mg) four times daily was also associated with a prolonged device life and lower intratracheal phonatory pressures.[8,27] One of the drawbacks encountered in the use of this agent is certainly the frequent daily applications, leading to poor compliance by the patients. Drug concentrations in saliva are important for efficacy in oropharyngeal candidiasis.

Application of bioadhesive tablets containing 10 mg of miconazole demonstrated a significant reduction of yeast colonization ($p < .001$; Friedman test) in the oropharynx and on the Provox voice prosthesis. At the end of this study, 94% of the patients in the active substance group had negative cultures. In contrast, 80% of the patients treated with placebo were found to have significantly higher colonization indexes ($p < .001$; Friedman test) of *Candida* spp at the valve prostheses. The majority of noncolonized subjects receiving miconazole remained free of fungal colonization during this trial. In 83% of noncolonized subjects who received placebo, a positive yeast culture was obtained after 1 month of prosthesis replacement. Unless high concentrations of yeast organisms were demonstrated in the tracheoesophageal shunt and oral cavity, no clinical symptoms were manifested. Adherence of *Candida* spp is preferentially found on silicone materials, as the surrounding tissues are most often not involved.[5] Remarkably higher colonization indices in the placebo group were found at the valve prosthesis at 6 months compared to the fistula and oropharynx. Hence, microbial colonization of Provox prostheses with *Candida* spp may be prevented by using miconazole in a bioadhesive slow-release system.

Measurements of the intratracheal phonatory pressure at various intensity levels showed a significant increase ($p < .001$; Friedman test) in both groups. Stabilization of the intratracheal pressure increase was noticed in the miconazole group after 2 months of follow-up. However, a continuous increase of intratracheal phonatory pressure was recorded in subjects receiving placebo. Initial pressure rises in both groups may be provoked by influences of esophageal contents on the valve prosthesis. The pressure recordings correlated well with the gradual accumulation of deposits on the surface of the prostheses. In the placebo group a remarkable increase of nonremovable deposits was observed after 4 months of use. This was associated with higher colonization indexes and higher intratracheal phonatory pressures. The degree of fungal colonization of the silicone material seems to be correlated with the expiratory airflow resistance of the valve mechanism of the voice prosthesis.[8] This may gradually diminish the advantages of indwelling voice prostheses designed with a low-resistance valve mechanism to favor shunt speech production.[28,29]

High colonization indexes of yeast are associated with progressive dysfunction of valve prostheses. All subjects treated with bioadhesive tablets containing miconazole demonstrated a reduction of valve dysfunction and subsequent prosthesis replacements. After 1 year of follow-up, a remarkably longer device life was noticed in patients who received chemoprophylaxis. Compliance of the patients was satisfactory. This may be attributed to the absence of local adverse reactions to the bioadhesive system, and the limited daily dosages. Long-term intermittent or continuous use of antifungal drugs may induce antimicrobial resistance.[30] Resistance to oral azoles has been found in intensive care patients and other immunocompromised patients. Selective pressure exerted by antifungal agents on the microbiologic flora of patients may influence the prevalence and spectrum of fungal pathogens. However, during this study no evidence for development of resistance to miconazole was noticed. Intermittent short-term chemoprophylaxis may be reserved for the laryngectomee who is disturbed by frequent prosthesis replacement due to fungal deterioration.

Candida albicans is the one species most often associated with deterioration of valve prostheses. However, several other yeast species and other microorganisms can be cultured in the same individual over time.[23] Reduction of oropharyngeal yeast concentrations and inhibition of fungal adherence to silicone devices should be further investigated. Inclusion of antimycotics in the devices during manufacturing, and application of specific coatings, may be considered as possible options. Also, an altered polarization of the polymers may eventually be used to reduce the affinity of *Candida* spp for these materials.

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AUDIOLOGIC FINDINGS IN PATIENTS WITH A POINT MUTATION AT NUCLEOTIDE 3,243 OF MITOCHONDRIAL DNA

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A mitochondrial tRNA$^{Leu(UUR)}$ mutation at nucleotide 3,243 is known to be found in most patients with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes) and has also been identified in several families with maternally inherited diabetes mellitus and hearing loss. We report here audiologic features in patients with hearing loss associated with the mutation. Four patients without and five with MELAS were studied. Most of the patients had bilateral progressive sensorineural hearing loss. The most common shape of the audiogram was sloping, while cases in the advanced stages had flat audiograms. Speech discrimination scores were generally poor and did not parallel the degree of hearing loss. The present study suggests that the lesion for hearing loss could include both cochlear and retrocochlear involvement, but does not demonstrate a significant difference in the audiologic findings between patients with and without MELAS.

KEY WORDS — diabetes mellitus, MELAS syndrome, mitochondrial DNA, sensorineural hearing loss.

INTRODUCTION

The importance of mitochondrial DNA (mtDNA) in human pathology has become apparent in recent years. Specific mtDNA point mutations have been found to be associated with several distinctive syndromes of mitochondrial encephalomyopathies characterized by neurologic dysfunction and abnormalities in mitochondrial structure. One of these, an adenine to guanine transition at nucleotide 3,243 in the transfer (t)RNA$^{Leu(UUR)}$ gene of mitochondrial DNA, was found[1,2] and has been demonstrated to be present in a high proportion of patients with clinical features of MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes).[1-4] Hearing loss is an important feature in mitochondrial encephalomyopathies and was reported to be seen in about 30% of patients with MELAS and the mutation.[4]

On the other hand, the same mutation also has been found in pedigrees showing maternally inherited diabetes mellitus (DM) and sensorineural hearing loss.[5,6] More than half the patients with DM and the mutation, but without typical manifestations of MELAS, were reported to have hearing loss.[7] However, detailed audiologic descriptions of patients with the mitochondrial tRNA$^{Leu(UUR)}$ mutation at nucleotide 3,243 have not been reported. To characterize the audiologic features associated with the mtDNA mutation at nucleotide 3,243, we studied patients with hearing loss and the mutation, including those with DM but without MELAS, and those with MELAS. Possible audiologic differences between these two groups were analyzed.

MATERIALS AND METHODS

By the end of 1994, nine patients in our department with hearing loss had been demonstrated to have the mitochondrial tRNA$^{Leu(UUR)}$ mutation at nucleotide 3,243. Because of the additional restriction site of the endonuclease *Apa* I, the mutation was detected by amplifying the relevant segment of the mtDNA extracted from blood with polymerase chain reaction, followed by digestion with *Apa* I and separation of digested fragments from undigested ones.[1,2,8] The mutation was confirmed by DNA sequencing of the polymerase chain reaction products in two patients. The patients were classified into two groups: patients who had DM and hearing loss but did not have symptoms of MELAS (patients 1 through 4, group 1), and patients who had typical or clinically incomplete MELAS syndrome (patients 5 through 9, group 2).

All nine patients underwent general medical and

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CLINICAL AND AUDIOLOGIC FINDINGS IN PATIENTS WITH MITOCHONDRIAL DNA MUTATION STUDIED

	Patients in Group 1 (Without MELAS)				*Patients in Group 2 (With MELAS)*				
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Age at examination	42	52	50	45	17	14	20	44	35
Sex	F	F	F	F	M	F	F	M	M
Age at onset of hearing loss	39	45	40	18*	12	14	17	30	24
Tinnitus	–	+	+	+	–	–	+	–	+
Diabetes mellitus	+	+	+	+	–	–	+	+	+
Age at diagnosis	23	46	41	40			22	35	27
MELAS	–	–	–	–	±†	+	+	+	+
Age at diagnosis					17	11	16	42	33
Pure tone average (R/L, dB)	28/29	54/58	58/54	83/78	36/38	40/39	40/45	56/51	66/63
Shape of audiogram	Sharply sloping	Sloping	Sloping	Flat	Sharply sloping	Sloping	Sloping	Sloping	Flat
Maximal speech discrimination score (R/L, %)	68/70	66/72	62/50	16/18	62/64	80/82	66/68	62/56	36/64
Reflex threshold average (R/L, dB HL)§	90/87	93/92	102/100	No reflex	94/95	98/101	96/100	103/105	111/111
Jerger's type of Békésy audiometry	II	II	I	I	I	U	NA	I	II
ABR latency	NA	P	N	NA	N	N	P	P	N
Caloric response	N	N	N	C	N	N	C	NA	NA

U — unclassified, NA — not available, P — prolonged latency, N — normal, C — canal paresis.

*Hearing loss due to chronic otitis media.

†He had some symptoms of MELAS, but did not fulfill diagnostic criteria.

§Reflex threshold average calculated as average of 0.5, 1, 2, and 4 kHz in each ear.

otologic examinations. Complete audiologic examinations included pure tone thresholds obtained with a Rion AA-61BN audiometer, speech discrimination scores with TY-89 Japanese word lists, tympanometry and acoustic reflex thresholds with a Dana Japan DANAC 45, Békésy audiometry with a Rion RE-04, auditory brain stem response (ABR) with a NEC Sanei Synax ER 1100, and bithermal caloric testing. The pure tone average (PTA) levels were calculated as the average of air conduction thresholds at 0.5, 1, 2, and 4 kHz in each ear. Changes in PTA were evaluated in cases with more than a 36-month follow-up. In this study, the shape of the pure tone audiogram was categorized into one of three types based on the classification described by Paparella et al[9]: a flat audiogram, in which the difference between the PTA levels of low (0.125 and 0.25 kHz), middle (0.5, 1, and 2 kHz), and high (4 and 8 kHz) frequencies is less than 10 dB; a sloping audiogram, in which the difference between the averages of the middle and high frequencies is 10 to 30 dB; and a sharply sloping audiogram, in which the difference between the averages of the middle and high frequencies is greater than 30 dB. Other otologic problems that may cause hearing loss, such as chronic middle ear disease, infection, and ototoxic agents, also were evaluated.

RESULTS

Clinical and audiologic findings are summarized in the Table. In group 1, the only significant clinical manifestation other than DM and deafness was a history of angina pectoris in patient 2. None of these patients had ever had a history of ketoacidosis. In patients 2 and 3, DM was well controlled with diet and oral hypoglycemic agents. Patients 1 and 4 had been treated with diet and oral hypoglycemic agents until they required insulin therapy, at the ages of 32 and 42 years, respectively. Group 2 included two maternal relatives. Patient 9 was the sibling of patient 4, and patient 5 was the son of patient 3. Four patients (6 through 9) fulfilled the diagnostic criteria for MELAS.[3,4] Patient 5 had some of the common symptoms of MELAS such as short stature, mental retardation, and high serum lactate concentrations, but had no strokelike episodes. This patient could be considered to have clinically incomplete MELAS,[3,10] although biochemical and morphologic data were unavailable. Other etiologic factors of hearing loss, apart from DM, were excluded in all cases with the exception of patient 4, who had chronic otitis media in the right ear and a history of right tympanoplasty at the age of 32 years.

The onset of hearing loss was compared between

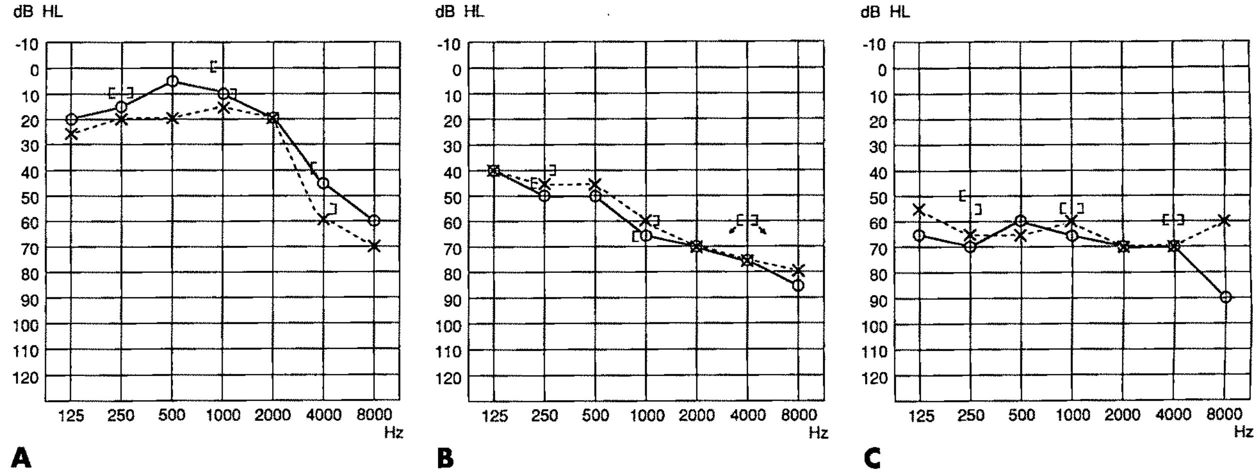

Fig 1. Examples of three types of pure tone audiogram shape. A) (Patient 1, age 42) Sharply sloping. B) (Patient 8, age 46) Sloping. C) (Patient 9, age 37) Flat.

group 1 (except patient 4) and group 2 by means of an unpaired *t*-test. Hearing loss appeared significantly earlier in the patients of group 2 than in those of group 1 ($p < .005$). All patients except patient 4 had bilateral sensorineural hearing loss with symmetric pure tone audiograms bilaterally. Differences in PTA levels between ears were less than 5 dB overall. Patient 4 had bilateral hearing loss of a mixed type. Examples of the three shapes of pure tone audiogram are presented in Fig 1. In each group, the patient with the best PTA level had a sharply sloping audiogram, while the patient with the poorest one had a flat audiogram. With respect to the shape of the audiogram, there were close intrafamilial resemblances between patients 3 and 5 and between patients 4 and 9. The course of hearing loss was found to be gradually progressive, but its progression rate varied among the cases (Fig 2). In general, speech discrimination scores were poor in comparison to pure tone thresholds. This proved true even in patients who had relatively mild degrees of hearing loss, such as patients 1 and 5. All nine cases had normal tympanograms. Acoustic reflexes were present in all except patient 4, who had severe hearing deterioration. Among the three with prolonged latencies for ABR, patient 2 showed a latency delay of waves III and V, and the others showed changes in wave V. Caloric response was diminished in one case in each group without complaint of vertigo.

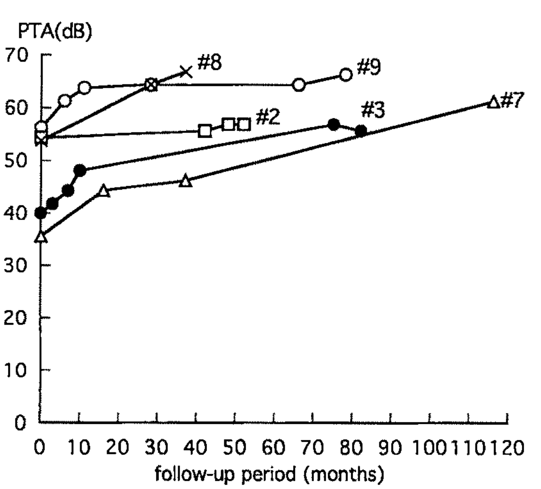

Fig 2. Course of hearing loss in patients followed more than 36 months.

DISCUSSION

A small number of reports have been published to describe the audiologic findings in mitochondrial disorders.[11-14] However, there is no detailed description of hearing loss associated with the mitochondrial $tRNA^{Leu(UUR)}$ mutation at nucleotide 3,243 in the literature. In this study, hearing loss associated with the mtDNA mutation at nucleotide 3,243 demonstrated some audiologic characteristics common to patients both with and without MELAS. First, the most common shape of the audiogram was sloping. The sharply sloping shape suggests that hearing loss in the earlier stages started at high frequencies, whereas patients in the advanced stages, who had a PTA level of more than 60 dB, revealed a flat audiogram. Second, speech discrimination scores, which deteriorated to various degrees, were generally poor in comparison to pure tone thresholds and did not parallel the degree of hearing loss. Third, the audiologic findings showed both cochlear and retrocochlear involvement, which differed considerably among patients.

The relationship between audiogram shapes and the degree of hearing loss within the present cases was consistent with that described in the mitochondrial disorders.[12-14] However, speech discrimination scores in this study were generally poor, whereas those described by Elverland and Torbergsen[13] were often 100%, even in patients who had a PTA level of more than 50 dB. They also found that hearing loss associated with a mitochondrial disorder had cochlear characteristics in mild cases, and that the more severely affected patients demonstrated retrocochlear signs. In the present study, however, patient 9 exhibited cochlear involvement despite his poorer PTA. These differences could partly be attributed to interfamilial variation. This could also account for the variable progression rate of hearing loss in this study. The rate was reported to be constant in the study by Elverland and Torbergsen.[13]

If it is true that DM has either a direct or an indirect influence on auditory function, it should be taken into account in audiologic studies. To date, there seems to be some disagreement about whether DM can cause hearing loss.[15,16] Japanese authors[7,17] have reported that hearing loss often develops after the onset of diabetes in patients with the mutation; the reverse also has been described.[18,19] In this study, the onset of hearing loss preceded that of DM in all but one case. Moreover, in some families with the mtDNA mutation at nucleotide 3,243, several instances of hearing loss unassociated with DM were found in the maternal relatives of patients with DM and hearing loss.[5-7] These facts suggest that the hearing loss associated with this mutation is not secondary to DM. Even if DM could affect hearing, it would be doubtful whether its effect could result in the variety of audiologic findings.

The diversity of the present findings might characterize hearing loss associated with the mutation. A variety of other clinical features in patients with the mutation were reported, even within a single family.[10,20] It is hypothesized that the phenotypic expression of a pathologic mutation of mtDNA might be mainly determined by the relative proportion of wild-type and mutant mtDNAs in each organ. In other words, each organ may have its own threshold for the proportion that impedes its function. This concept is known as the threshold effect.[21] Coexistence of wild-type and mutant mtDNAs in the same tissue is called heteroplasmy. It is conceivable that the expression of either cochlear or retrocochlear characteristics might reflect different degrees of mtDNA heteroplasmy in the inner ear and central auditory pathway.

The inner ear, with its high energy demands, is thought to be susceptible to metabolic damage from cell dysfunction caused by mutant mtDNAs. While no one has described the histopathologic features of the inner ear in patients with hearing loss associated with the mutation, Lindsay and Hinojosa[22] have reported findings in a case with Kearns-Sayre syndrome, which is usually associated with mtDNA deletions instead of mutation.[23] They have described advanced cochleosaccular degeneration, including the collapse of Reissner's membrane; degeneration of the organ of Corti, tectorial membrane, and stria vascularis; a considerable reduction in the number of spiral ganglion cells; and degeneration of nerve fibers within the osseous spiral lamina. Pathologic changes in the central auditory pathways have been reported, such as spongy or vacuolar changes in the cerebral white matter, deep gray matter, and brain stem nuclei, including the eighth cranial nerve nuclei.[24-26] These findings strongly suggest that both cochlear and retrocochlear pathologic changes can be caused by mitochondrial disorders. Moreover, in MELAS patients, mitochondrial angiopathy was observed in cerebral blood vessels.[27] If small vessels of the inner ear (such as those of the stria vascularis) or vessels associated with the central auditory pathway are involved, this may also play a role in the development of hearing loss.

Although the total number of cases was rather small, there were no significant differences in the audiologic findings between the two groups in the present study. Patients in group 2 with symptoms of MELAS were thought to have dysfunction of the central nervous system. Therefore, it was expected that the retrocochlear signs would predominate over the cochlear signs in the audiologic evaluation of these patients. However, even the MELAS patient with severe hearing loss manifested cochlear signs (as mentioned above). In addition, we could not find any differences in the rate of progression of the hearing loss between the two groups, although it is conceivable that MELAS is a severer presentation of the phenotypes associated with this mutation. However, our data are insufficient to warrant a conclusion as to whether there are any differences in audiologic features associated with the mutation between patients with and without MELAS. Elucidation of the mechanism of hearing loss, as well as the audiologic characteristics associated with this mutation, must await the accumulation of additional data.

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IMAGING CASE STUDY OF THE MONTH

VALUE OF FAT SUPPRESSION MAGNETIC RESONANCE IMAGING IN THE DIAGNOSIS OF LIPOMAS OF THE INTERNAL AUDITORY CANAL

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INTRODUCTION

Lipomas of the intracranial cavity, very rare tumors, are probably malformative rather than neoplastic in nature.[1] Approximately half of these lesions are located adjacent to the corpus callosum.[2] Lipomas involving the cerebellopontine angle (CPA) or internal auditory canal (IAC) are extremely rare, although preoperative diagnosis has been increasingly made with the advent of computed tomography (CT) and magnetic resonance imaging (MRI) since 1978.[3]

The CPA lipomas typically present with slowly progressive focal signs and symptoms. Christensen et al[4] proposed the most minimal excision of the tumor possible to achieve decompression, because dissection of the adipose tissue from the cranial nerves in the total removal of CPA lipoma led to thinning of the nerves, resulting in greater than anticipated neurologic deficits. Nevertheless, neurosurgical intervention has been tried to confirm the diagnosis of CPA lipoma.

Fat suppression techniques[5] have been used in MRI to suppress strong signals from fatty tissues that interfere with signals from adjacent areas. The purpose of this report is to present 2 cases of IAC lipoma, which brings, to our knowledge, the total cases so far reported in the English- and German-language literature to 35, and to elucidate the usefulness of the fat suppression techniques in MRI to confirm the diagnosis of CPA and IAC lipomas.

CASE REPORTS

Case 1. A 51-year-old man presented with a 10-year history of right hearing impairment. He also complained of right-sided tinnitus, but denied any vertigo, otalgia, or facial weakness. His audiogram revealed a right high-frequency sensorineural hearing loss, with a pure tone average of 42.5 dB (Fig 1), and a speech discrimination of 50%. His auditory brain stem response examination demonstrated no abnormality. No caloric response was obtained in the right ear with 20 mL of cold water (0°C), although the response was normal in the left ear. Axial T1-weighted (TR = 380, TE = 19.0 milliseconds) MRI (Signa 1.5 T, matrix = 256 × 192, 4.0 NEX) demonstrated a 5 × 10-mm hyperintense tumor in the right IAC. T2-weighted images (TR = 3,000, TE = 90.0 milliseconds) revealed a reduced signal intensity. The signal from the hyperintense tumor in the right

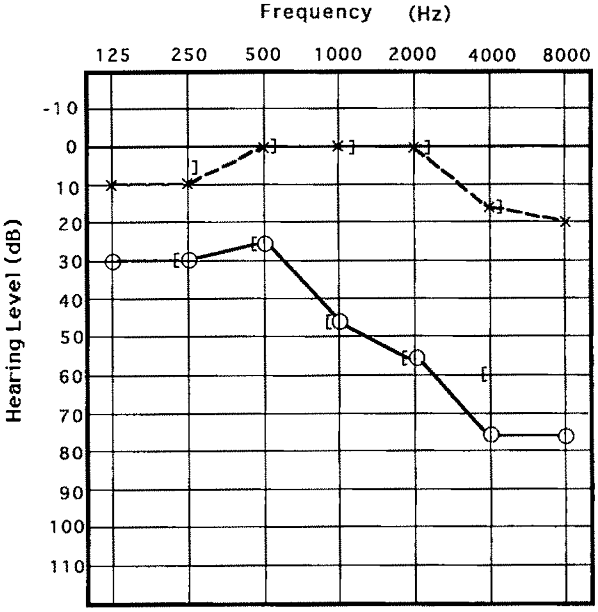

Fig 1. (Case 1) Audiogram performed November 8, 1993, showing sensorineural hearing loss at high frequencies with pure tone average of 42.5 dB on right.

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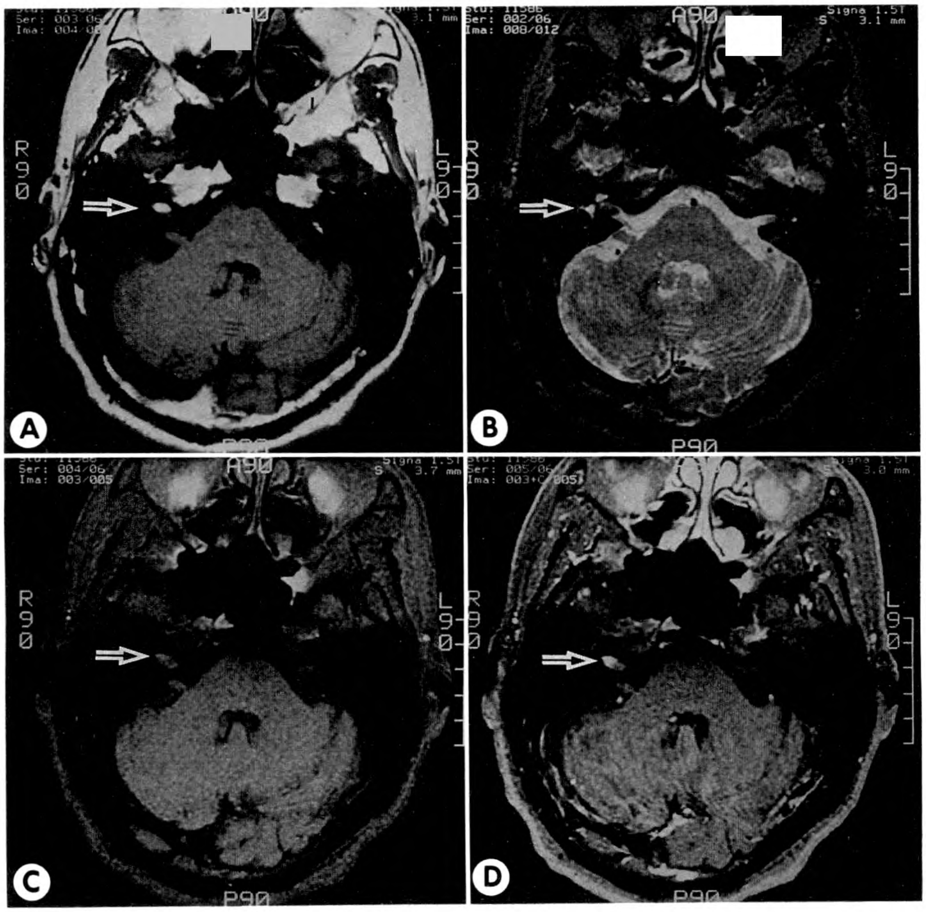
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Fig 2. (Case 1) Magnetic resonance imaging (MRI) findings. Arrows—tumor. A) T1-weighted images revealed hyperintense tumor 5 × 10 mm in diameter in right internal auditory canal (IAC). B) Signal from tumor in right IAC was hypointense on T2-weighted MRI. C) T1-weighted images with fat suppression. Hyperintense signal was markedly suppressed. D) Gadolinium enhancement was clearly demonstrated in fat-suppressed, T1-weighted MRI.

IAC was markedly suppressed by fat suppression (frequency-selective presaturation) in T1-weighted MRI. The gadolinium-enhanced and fat-suppressed T1-weighted MRI demonstrated a homogeneous enhancement of the IAC lesion (Fig 2). Follow-up audiograms have demonstrated no deterioration or fluctuation in his hearing level for the last 1$^1/_2$ years. The MRI examinations performed every 6 months have shown no change in the size of the tumor.

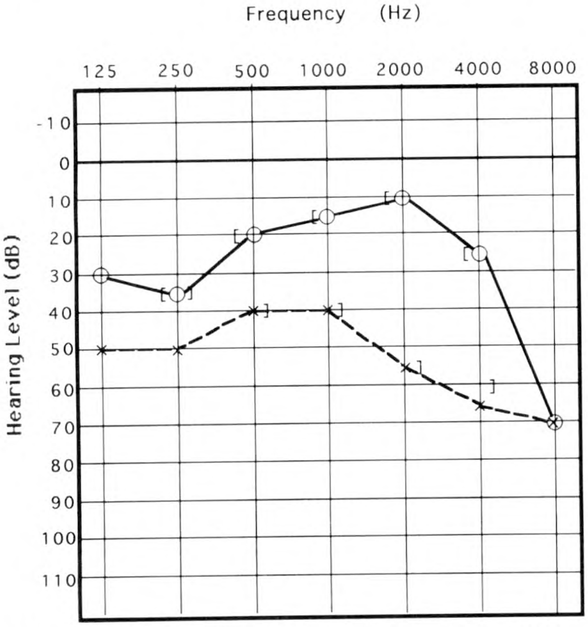

Fig 3. (Case 2) Audiogram performed August 18, 1992, revealing left high-frequency sensorineural hearing loss with pure tone average of 43.8 dB.

Case 2. A 49-year-old man was referred to our hospital with a 1-year history of progressive left-sided hearing loss. He denied any tinnitus, facial weakness, vertigo, or other symptoms. The findings on physical examination were normal except for the hearing loss. Audiometry showed a sensorineural hearing loss at high frequencies, with a pure tone average of 43.8 dB on the left (Fig 3). His auditory brain stem response test demonstrated prolonged latencies of the I-III or I-V wave on the left side consistent with a retrocochlear lesion. The caloric test revealed a canal paresis in the left ear; the maximum slow-phase velocities were 42°/s and 24°/s at 44°C, and 29°/s and 13°/s at 30°C in the right and left ears, respectively. The MRI (Signa 1.5 T) revealed a 7 × 9-mm mass in the left IAC. On T1-weighted MRI (TR = 380, TE = 19.0 milliseconds), the IAC mass emitted a hyperintense signal. On T2-weighted MRI (TR = 3,000, TE = 90.0 milliseconds), the lesion had an isointense signal. The signal from the lesion in the left IAC on T1-weighted MRI was

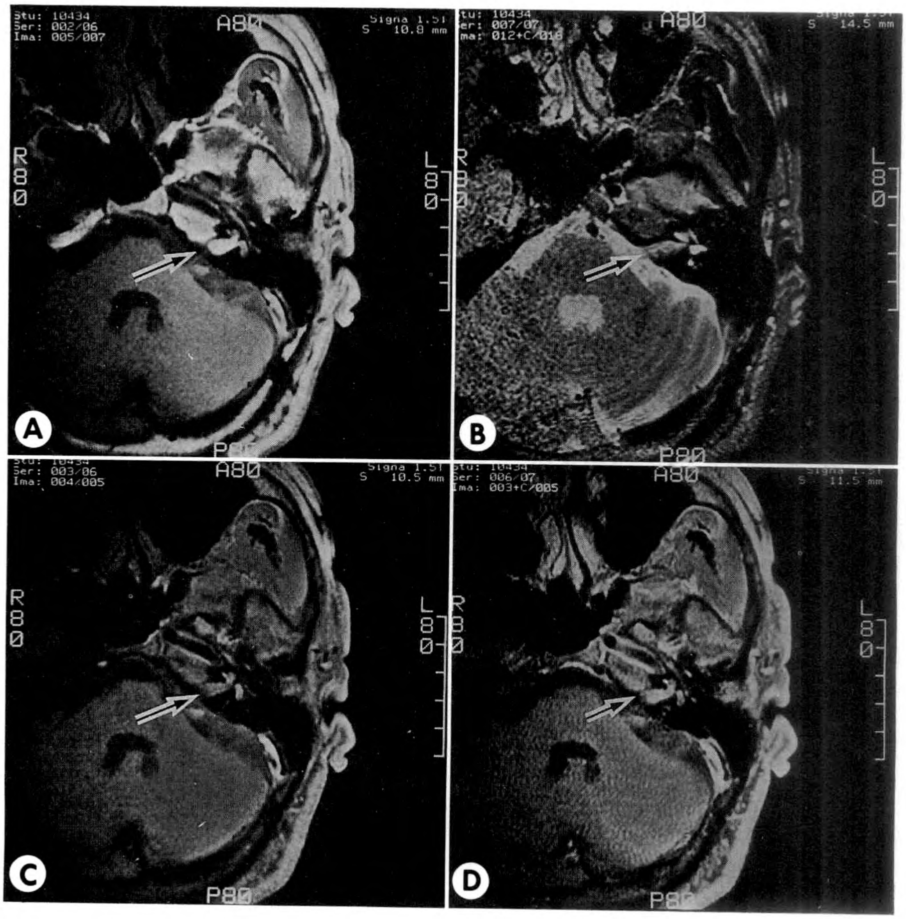

Fig 4. (Case 2) MRI findings. Arrows — tumor. A) T1-weighted MRI. Hyperintense tumor 7 × 9 mm in diameter was recognized in left IAC. B) Tumor had isointense signal on T2-weighted MRI. C) Hyperintense signal from tumor in left IAC on T1-weighted MRI in A was suppressed (isointense) on fat-suppressed, T1-weighted images. D) Gadolinium-enhanced, T1-weighted MRI with fat suppression. Tumor was enhanced in comparison with fat-suppressed T1-weighted image in C.

suppressed by a fat suppression technique. The gadolinium-enhanced and fat-suppressed T1-weighted MRI revealed a bright signal within the left IAC (Fig 4). During the follow-up period of 2½ years, audiometry demonstrated no change in his hearing level, and MRI showed no increase in the size of the tumor.

DISCUSSION

Lipomas in the CPA and IAC are extremely rare, and only 33 cases, to our knowledge, have been reported in the English- and German-language literature since the first case of a CPA lipoma found at autopsy was reported by Klob[6] in 1859. A summary of the 33 cases and our present 2 cases is shown in Table 1.[3,4,6-26] Lipomas of the CPA affect males more commonly (male to female ratio 2.8 to 1), with no age predominance, and occur in the left CPA (24 cases) more often than the right (9 cases). In contrast to most other intracranial lipomas, CPA lipomas are often symptomatic. Eighty percent of 30 cases described in the literature demonstrated hearing loss, and more than 40% had tinnitus and/or vertigo. Accordingly, the clinical presentation is often indistinguishable from that of an acoustic schwannoma.[19] Kitamura et al[24] reported 2 cases of CPA lipoma with fluctuating hearing loss, although the audiograms of our 2 patients revealed threshold shifts of 10 dB or less.

Radiographic findings previously reported are also shown in Table 1. Polytomography revealed the erosion of IAC in 9 of 10 cases.[9,11-15,17,19,24,26] A hypodense lesion was recognized in 14 (87.5%)[3,4,14-22,25] of 16 cases undergoing CT examinations, although an acoustic schwannoma–like lesion was recognized in 1 case,[4] and in another lesion was undetectable on CT except for the bone erosion within the IAC.[26] Since the first report by Levin and Lee[20] in 1987, 11 cases in the CPA have been clearly demonstrated with MRI findings. Typical MRI findings are a hyperintense signal on T1-weighted images (100%)[20-22,24-26] and a hypointense (44%),[24,26] isointense (33%),[26] or hyperintense (22%)[20,21] signal on T2-weighted images. The enhancement of the intensity on T1-weighted images with gadolinium was recognized in all of 5 cases.[26]

The cause of CPA lipoma is still controversial, although it is not a neoplasm.[1] Budka[9] reiterated that a rapid growth or a malignant transformation with metastasis had not been observed. Lipomas of the CPA typically present slowly, with progressive focal signs and symptoms.[4] The duration of symptoms of the 35 cases ranged from 5 months to 10 years, with a mean of 4.4 years (Table 1).

TABLE 1. SUMMARY OF REPORTED CASES OF CEREBELLOPONTINE ANGLE LIPOMA

No. of cases	35
Age	
Range	10 mo to 77 y
Mean	40.5 y
SD	17.0 y
Male to female ratio	25:9
Side	
Left	24
Right	9
Bilateral	1
Symptoms (n = 30 patients)	
Hearing loss	24 (80%)
Vertigo	12 (40%)
Facial palsy	4 (13.3%)
Facial spasm	2 (6.7%)
Tinnitus	13 (43%)
Pain	6 (20%)
Headache	3 (10%)
Seizure	1 (3.3%)
Time to diagnosis (n = 24 patients)	
Range	5 mo to 10 y
Mean	4.4 y
SD	3.8 y
Radiographic findings	
Polytomography	
Erosion of IAC	9/10 (90%)
Computed tomography	
Hypodense lesion	14/16 (87.5%)
Neurinoma-like lesion	1/16 (6.3%)
Erosion of IAC	1/16 (6.3%)
Magnetic resonance imaging	
T1W1 high-intensity	11/11 (100%)
T2W1 low-intensity	4/9 (44.4%)
T2W1 isointense	3/9 (33.3%)
T2W1 high-intensity	2/9 (22.2%)
Gd enhancement	5/5 (100%)
Diagnosis	
Autopsy	6/35
Imaging studies	5/35
Surgical specimen	24/35
Total removal	9/24 (37.5%)
Partial or subtotal	13/24 (54.2%)
Biopsy	2/24 (8.3%)
Outcome	
Hearing loss	
Cranial nerve VIII	20/24 (83.3%)
Paresis	
Cranial nerve VII	10/24 (41.7%)
Cranial nerve X	4/24 (16.7%)
Cranial nerve V	3/24 (12.5%)
Others	4/24 (16.7%)

Based on present cases and information in references 3, 4, 6-26.
IAC — internal auditory canal, Gd — gadolinium.

TABLE 2. MAGNETIC RESONANCE CHARACTERISTICS OF CEREBELLOPONTINE ANGLE LESIONS

	T1	*T1-Gadolinium*	*T2*	*Fat Suppression*
Acoustic schwannoma	↓	↑	↓↑	
Meningioma	↓↑	↑	↓↑	
Epidermoid	↓	→	↑	
Arachnoid cyst	↓	→	↑	
Metastasis	↓	↑	↑BE (+)	
Lipoma	↑	↑E (+)	↓→	
Case 1	↑	↑E (+)	↓	↓
Case 2	↑	↑E (+)	→	↓

↓ — hypointense, ↑ — hyperintense, → — isointense, BE — brain edema, E — enhancement, + — positive.

Furthermore, the outcome of surgical intervention previously reported is rather poor, with a high incidence of postoperative cranial nerve deficits (Table 1). Christensen et al[4] proposed that the surgery be restricted to the most minimal excision of the tumor to confirm the diagnosis and to achieve decompression, because of the tendency of CPA lipoma to splay apart and infiltrate the cranial nerves. In fact, total removal of lipoma was performed in only 9 (37.5%)[4,12,14,26] of 24 patients receiving surgical interventions (Table 1).

To prevent the postoperative neurologic deficits in patients with CPA lipomas and to confirm the diagnosis before or without surgical intervention, confirmative imaging studies are essential. A very low-density mass on CT is the most suggestive finding, but even this does not definitely exclude a possibility of acoustic neurinoma[4] or cholesteatoma, as shown in Table 1. The characteristics of MRI in the patients with CPA lesions reported by Lalwani[27] and in our 2 cases are summarized in Table 2. A hyperintense signal on T1-weighted images is characteristic of CPA lipoma. In fact, MRI in all 11 cases reported so far has revealed the hyperintense lesions of CPA without exception (Table 1). On the other hand, a hyperintense signal on T1-weighted images can be recognized in epidermoid or acoustic schwannoma with fatty degeneration. A fat suppression technique, therefore, is important in distinguishing a lipoma from other CPA lesions. Although many fat suppression techniques (short T1 inversion recovery, Dixon, hybrid, and other methods) are available, the frequency-selective presaturation method[5] was used in our cases. Hydrogen protons in fat precess at a frequency lower than water protons. This property, called chemical shift, allows the selection of the correct transmission frequency so that fat can preferentially first be excited and then be suppressed. With this technique, a frequency-selective presaturation pulse is applied to fat immediately before the excitation pulse, destroying the longitudinal magnetization of the fat. When the excitation pulse is applied, sufficient time will not have elapsed to allow significant longitudinal recovery of the fat signal, so little or no magnetization will occur. In our 2 cases, the hyperintense signals on T1-weighted MRI were completely and homogeneously suppressed (Figs 2 and 4) by this method. Gadolinium enhancement, furthermore, was clearly confirmed on the gadolinium-enhanced and fat-suppressed T1-weighted MRI in both cases, although it might be difficult to judge whether the lipoma is enhanced or not, because it has already demonstrated a hyperintense signal on T1-weighted MRI unless the signal is suppressed by a fat suppression technique.

This is the first report describing MRI findings applying a fat suppression technique in patients with CPA lipomas. The results suggest that MRI with this technique is meaningful and useful in confirming the diagnosis of CPA lipoma, and even in avoiding postoperative morbidity in patients without severe or progressive symptoms.

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CLINICOPATHOLOGICAL CONSULTATION

NASOPHARYNGEAL CARCINOMA, WITH EMPHASIS ON ITS RELATIONSHIP TO EPSTEIN-BARR VIRUS

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Nasopharyngeal carcinoma (NPC) is an epithelial tumor with a distinct geographic distribution and a characteristic histologic appearance. It is rare in Europe and North America, but it is among the most common cancers in southern China. Genetic predisposition, environmental factors, and Epstein-Barr virus (EBV) all have been associated with the pathogenesis of this tumor. There is an increasing body of evidence that among all these factors, EBV appears to be the strongest and most consistently related factor. According to the current sensitive in situ hybridization methods for the detection of EBV-encoded small RNAs (EBER), almost 100% of cases of NPC, irrespective of their histologic subtypes, have demonstrable EBERs in the nuclei of the tumor cells. In this review paper, we discuss the predisposing genetic and environmental factors and the role of EBV in the pathogenesis of this tumor with particular emphasis on the role of EBV.

Nasopharyngeal carcinoma (NPC) is distinct from other carcinomas of the head and neck, with a characteristic geographic distribution, unique etiologic factors, and an often characteristic histologic appearance.

EPIDEMIOLOGY

Nasopharyngeal carcinoma is rare in most countries of the world. However, there is an exceptionally high incidence of this neoplasm in the southern Chinese province of Guangdong (Canton) and Hong Kong,[1-4] with a yearly incidence rate between 10 and 50 per 100,000. Sometimes NPC is called "Cantonese cancer." In Taiwan, this neoplasm accounts for approximately 42% of all head and neck tumors treated in otolaryngology departments[3] and is the most common cancer in males and the third most common cancer in females.[5] The incidence is also high among Eskimos in Alaska and Greenland, varying between 15 and 20 per 100,000.[4,6] The disease has an intermediate incidence rate in northern China, southeast Asia, northern Africa, southern Italy, Greece, and Turkey.[7,8] The incidence remains elevated among first-generation Chinese who have migrated to other parts of the world, while the risk of developing NPC decreases in the second generation.[9] The disease has been reported in all age groups, ranging from 9 to 75 years.[3] In high-risk areas, most patients are in their 40s. The disease is predominantly seen in males, with a male-female ratio of 3:1.

In most whites in Europe and North America, Japanese, and Asian Indians, a low incidence is seen (about 0.5 to 1.0 per million).[10] In the Western world, NPC accounts for only 0.25% of all malignant tumors.[11] Among white male patients in the United States with NPC, a broad age distribution is observed, with a peak at 20 to 29 years and a second peak at 60 to 69 years.[12] Black patients have a lower-incidence peak between 10 and 19 years.[12]

PATHOLOGY

Nasopharyngeal carcinoma is derived from nasopharyngeal surface epithelium, particularly in Rosenmüller's fossa, and this location is actually the meeting point of the lateral wall and the posterior wall.[13]

Histologically, NPC is subclassified into three major groups according to the WHO proposal[14]: keratinizing squamous cell carcinoma, nonkeratinizing squamous cell carcinoma, and undifferentiated nasopharyngeal carcinoma (UNPC). Keratinizing squamous cell carcinoma is the well-differentiated subtype showing keratinization and/or easily identifiable intercellular bridges and represents about 20% of cases.[14,15] In nonkeratinizing squamous cell carcinoma, the tumor cells lack light microscopic evidence of squamous cell differentiation, but there are well-defined cell margins and a pavement stone pattern. This subtype represents about 40% of cases.

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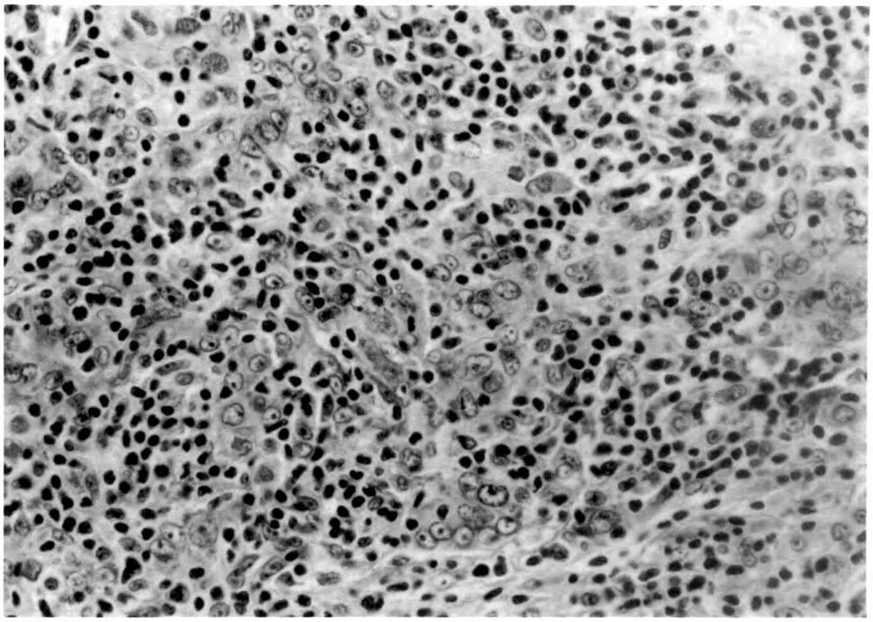

Fig 1. Undifferentiated nasopharyngeal carcinoma (H & E, original ×400). Tumor cells are dispersed singly or in small clusters in abundant lymphoid stroma. This histologic pattern of nasopharyngeal carcinoma may be difficult to distinguish from lymphoma without immunohistochemical stains.

Undifferentiated NPC shows syncytial aggregates of tumor cells with an intense lymphoplasmacytic infiltrate (Fig 1). The neoplastic cells in this subtype of NPC are cytologically uniform and have round to oval vesicular nuclei and prominent nucleoli. The cell borders are indistinct and there is no evidence of mucin production or glandular differentiation.[15] This pattern represents about 40% of cases of NPC. Two microscopic patterns have been described: the Regaud pattern,[16] in which well-defined, cohesive tumor aggregates and cords are separated by an inflammatory infiltrate with a predominance of lymphocytes; and the Schmincke pattern,[17] in which the inflammatory cells permeate the tumor nests and create isolated tumor cells surrounded by a dense inflammatory infiltrate composed predominantly of lymphocytes. The UNPC and nonkeratinizing squamous cell carcinomas (WHO type 2) with abundant lymphoid cells in the stroma have also been called lymphoepithelioma and lymphoepithelial carcinomas. The nonneoplastic lymphoid infiltrate may on occasion obscure the epithelial nature of the tumor and may be confused with malignant lymphoma on histologic evaluation, particularly with the clinical presentation of bilateral lymphadenopathy. In these cases, the diagnosis can easily be established by demonstration of keratin in the neoplastic cells by immunohistochemical stains[18-22] (Fig 2). Positivity for epithelial membrane antigen and negativity for leukocyte common antigen contribute further proof of epithelial

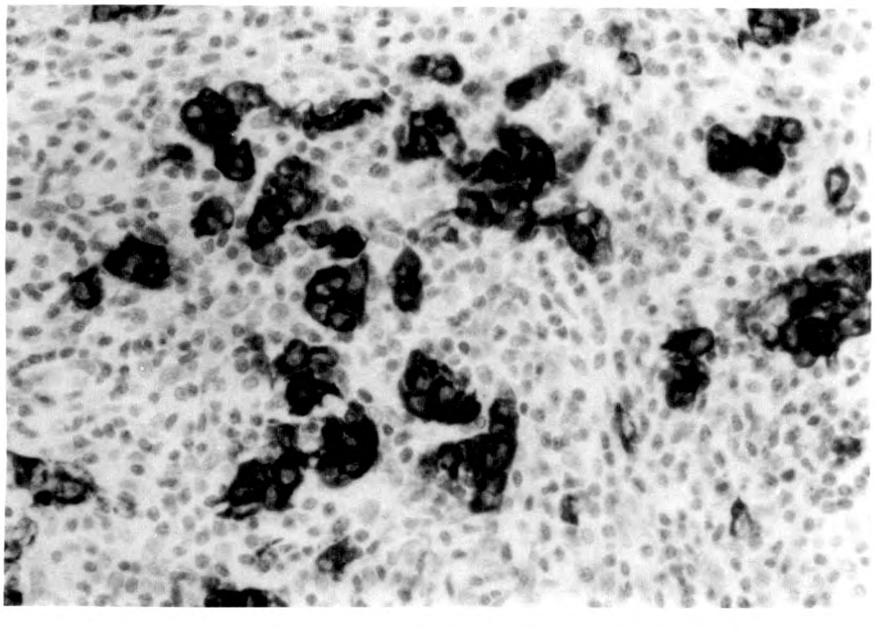

Fig 2. Undifferentiated nasopharyngeal carcinoma cells expressing keratin (immunoperoxidase and hematoxylin counterstain, original ×400).

differentiation.

Preinvasive lesions such as various degrees of dysplasia and carcinoma in situ may be seen adjacent to invasive carcinoma. Preinvasive lesions without invasive carcinoma in adjacent tissue have also been noted in less than 1% of cases.[23] Some of these preinvasive lesions have progressed to frankly invasive carcinoma at follow-up examination.[23] However, due to the lack of long-term follow-up of large numbers of these preinvasive lesions, the natural history of these lesions is not as yet entirely defined.

CARCINOGENESIS

The pathogenesis of NPC has been the subject of intense investigation. Genetic predisposition, environmental factors, and Epstein-Barr virus (EBV) have been linked to the pathogenesis of the disease. The increased risk for NPC in Chinese has been associated with certain Chinese-related HLA antigen profiles (inherited HLA-A2, HLA–Singapore 2 on the same chromosome, and fewer than two antigens at the B locus).[24-27] Alcohol and tobacco appear to have no etiologic role; however, occupational exposures to fumes, smoke, and chemicals have been linked to NPC.[26] In addition, ingestion of salted fish has been associated with an increased risk for NPC.[28-30] It has also been suggested that the presence of specific lignin fractions in high-risk food items can activate the lytic replication of EBV, and this may be important in the development of NPC.[31]

Cytogenetic abnormalities, especially consistent, nonrandom deletions of the short arm of chromosome 3 (loss of heterozygosity) at two chromosomal loci (3p25 and 3p14), have been detected by using restriction fragment length polymorphism analysis, suggesting the presence of tumor suppressor genes at these loci.[32,33] Abnormalities of other chromosomes have also been reported.[34]

Evidence of p53 overexpression has been found in a majority of cases of UNPC by immunohistochemistry.[35] Nonetheless, point mutation of the p53 gene is only a rare finding in both primary and metastatic tumors.[36,37] Overexpression of the bcl-2 oncogene product has been found in about 80% of cases of UNPC.[38]

VIROLOGY

Epstein-Barr virus is a member of the herpes family, Herpesviridae.[39] The virus was originally discovered in 1964 by Epstein et al[40] in cultured lymphoblasts obtained from patients with Burkitt's lymphoma. It has been found to be a major etiologic agent of acute infectious mononucleosis and has been associated with a wide variety of lymphoid neoplasms, including endemic Burkitt's lymphoma, posttransplantation lymphoproliferative disorders, a significant subset of Hodgkin's disease, and nasal T-cell lymphoma.[41] The virus consists of three components: a nucleoid, a capsid, and an envelope.[40,42,43] The viral DNA is double-stranded and is present in the core of the virus in condensed form.[44] This core is surrounded by a capsid composed of tubular protein subunits called capsomers.[43] The viral envelope that is derived from host cell membrane surrounds the viral nucleocapsid.[45] The viral genome has been cloned and entirely sequenced. The genome shows either a linear or a circular (episomal) configuration. In the linear form, it is composed of a variable number of terminal repeats of about 500 base pairs, a unique sequence of about 10 kilobases, an internal repeat sequence of about 40 kilobases, a long unique region of about 120 kilobases, and another set of terminal repeats. Circularization occurs by fusion of the two terminal repeat regions.[46] The virus can be cultivated in vitro only in B lymphocytes and some human epithelial cells.[46]

NASOPHARYNGEAL CARCINOMA AND EPSTEIN-BARR VIRUS

The first link between NPC and EBV was the observation in 1966[47] that sera from patients with NPC contain precipitating antibodies directed against EBV-infected cells. Many studies performed in different countries have demonstrated elevated levels of IgG and IgA antibodies directed against viral capsid antigen (VCA) and the diffuse component of the early antigen (EA) in patients with NPC.[48-51] In contrast, such profiles are uncommon in patients with carcinomas at other sites of the head and neck. The IgA anti-VCA antibody response is the most specific for NPC,[49] and can be easily detected in 80% to 85% of cases of NPC by an indirect immunofluorescence test.[52] Serologic evaluation of these antibodies may prove useful as a screening method for the detection of the disease, particularly in high-risk areas.[51] Patients with NPC who respond well to therapy show declines in the antibody titers, while increases in the titer are associated with disease progression.[53,54] In addition to antibodies to VCA and EA, increased levels of IgA antibodies directed against EBV nuclear antigen (EBNA)–1 have been found in about 90% of NPC patients, in contrast to 7% of normal individuals and 14% of patients with other cancers; this finding suggests that this ELISA test may become a useful screening assay complementary to the more commonly used IgA-VCA immunofluorescence test.[48] Recently, IgG antibodies to the EBV replication activator (ZEBRA) have been found in patients with

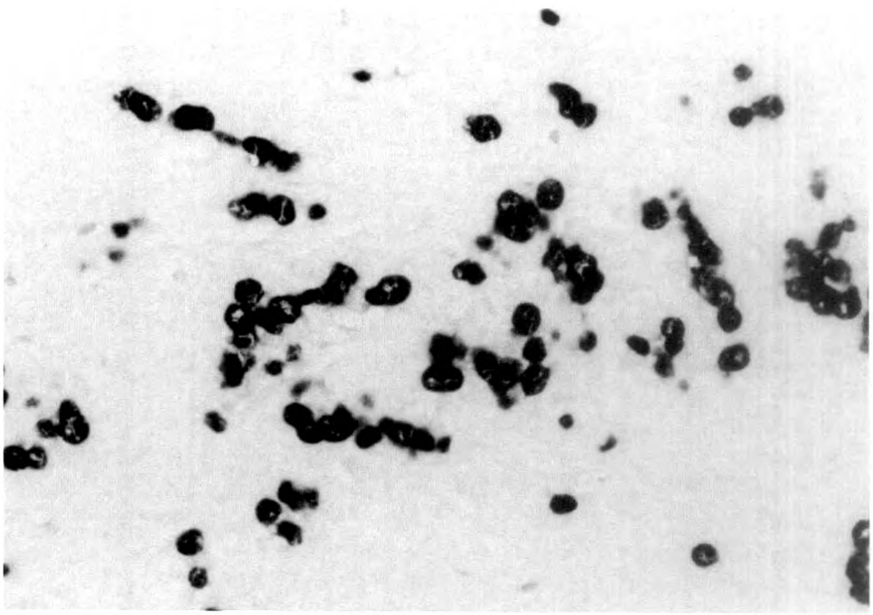

Fig 3. Undifferentiated nasopharyngeal carcinoma. Abundant Epstein-Barr virus RNA is evident within nuclei of neoplastic cells (in situ hybridization for virus-encoded small RNAs without counterstain).

NPC. Also detectable by an ELISA assay, IgG anti-ZEBRA antibodies can be identified in 25% of cases of NPC in which the IgA VCA was negative.[55] Determination of the titers of this antibody may have prognostic value in differentiating NPC patients with poor prognosis from those at high risk for the development of distant metastasis.[56]

Serologic evidence of EBV infection in NPC patients, of course, does not provide direct evidence for the presence of EBV in the tumor cells. In the early 1970s, EBV nucleic acid hybridization studies were used to directly demonstrate EBV genomes within tumor tissue.[57-60] Direct evidence for the presence of EBV within tumor cells and not passenger lymphocytes came in 1974, with the demonstration of EBNA-1 in the neoplastic cells.[61] A decade later, EBV DNA was demonstrated in the nuclei of the neoplastic cells by in situ hybridization.[62,63] Currently, the most sensitive and specific method for the identification of EBV in specific cells is the in situ hybridization for EBV-encoded RNA (EBER).[41,64,65] These EBERs are highly transcribed in all latently infected cells, with up to 10^6 copies in EBV-infected cells. Several groups have demonstrated EBER in NPC.[64,66]

Early studies found evidence of EBV in the majority of cases of NPC, particularly the UNPC variant. Nonetheless, the strength of the association, and whether the association also extended to the other variants of NPC, remained controversial. For example, one DNA in situ hybridization study demonstrated EBV in about 90% of UNPCs but failed to identify EBV in any case of squamous cell NPC,[67] while a Southern blot study demonstrated EBV in all of 33 cases of NPC, including 24 cases of UNPC, 4 "partially differentiated" tumors, and 5 well-differentiated NPCs.[68] Low copy numbers of EBV were identified in the latter cases, suggesting that lack of sensitivity may have accounted for the negative cases obtained in the other study. In situ hybridization studies of EBER have clarified the situation (Fig 3), with evidence for EBV RNA demonstrated in 120 of 120 cases of NPC, including 31 cases of keratinizing squamous cell carcinoma.[66]

In addition to identification of EBV in invasive tumor, in situ hybridization studies have also demonstrated EBER in preneoplastic lesions. Carcinoma in situ, whether associated with concurrent invasive tumor or microinvasion, or unassociated with invasion, is consistently EBV-positive.[23,69] Of interest, in many cases, the areas of carcinoma in situ are surrounded by an intense lymphocytic infiltrate. In addition, dysplasia, whether or not associated with concurrent invasion, is also consistently EBV-positive. Furthermore, EBV can occasionally be identified in apparently normal, hyperplastic, or metaplastic epithelium adjacent to invasive tumors.[69] However, EBV cannot be identified in biopsies showing normal nasopharyngeal histology from patients without NPC.[70] These studies suggest that EBV infection occurs at an early stage of carcinogenesis, prior to the development of invasive carcinoma. Therefore, screening nasopharyngeal biopsies for EBER may be useful in early NPC diagnosis.

Southern blot studies to determine the clonality of the cells harboring EBV in these lesions also support a role for EBV in the pathogenesis of NPC. One can use a probe to a segment of EBV genome near the terminal repeat region to determine whether the EBV infected a single progenitor cell that then underwent clonal expansion or whether the EBV entered several or more cells as an independent event.[71] Such studies have demonstrated that the EBV DNA in invasive NPC tumors is indeed present in a clonal population of cells. Even more interesting, 6 of 7 preinvasive lesions tested for the EBV termini were found to contain clonal EBV DNA, consistent with the hypothesis that EBV infection is a very early, and possibly initiating, event in the development of NPC.[23]

Epstein-Barr virus can be subtyped as type A (or 1) or B (or 2), based on differences in the organization of the EBNA-2 and EBNA-3A-C genes. Type A has a greater capacity to transform B lymphocytes in vitro compared to type B. Both types A and B can be isolated from oropharyngeal epithelial cells. Virtually all cases of NPC in southern China and Southeast Asia contain type A EBV.[33,72] Most cases of NPC from the Mediterranean, Africa, and the continental United States are type A, with some type B; while cases from Alaska are usually type B, despite containing polymorphisms characteristic of the Asian type A.[72]

Epstein-Barr virus infection of cells can be lytic (active) or latent. In a lytic infection, a wide range of EBV proteins are expressed, eventually leading to death of the cell. In a latent infection, only a limited range of proteins are expressed, including EBNA-1, EBNA-2, and EBNA-3A-C, leader protein, and latent membrane protein (LMP)–1, LMP-2A, and LMP-2B. EBNA-1 is essential to maintenance of the latent state and is presumably expressed in all latent infections, while expression of the other proteins varies in different neoplasms. For example, EBV-positive Burkitt's lymphoma cells have a latency pattern I, in which only EBNA-1 can be detected, while EBV-associated posttransplantation lymphoproliferative disorders have a latency pattern III, in which all the above-mentioned proteins are expressed.

The cells of NPC have a latency pattern II, in which EBNA-1 and the LMPs are usually expressed, but the other EBNA proteins are not.[73-77] Expression of LMP-1 has been found in 65% of cases of invasive tumor. Studies of preinvasive lesions of NPC have shown a similar pattern of latency, and although the number of cases studied is small, uniform expression of LMP-1 has been observed.[23] This pattern of latency is similar to that of Hodgkin's disease.[41] As discussed above, the ZEBRA protein (which is coded by the BZLF1 gene) is responsible for the switch from a latent to a lytic infection; therefore, detection of this protein or the transcribed gene would provide evidence for a lytic in addition to a latent infection. The RNA transcripts of the BZLF1 gene have been identified in NPC tumor cells by in situ hybridization.[78] In addition, expression of several other lytic genes has been found, suggesting that an abortive lytic cycle occurs in most cases of NPC, thus explaining the detection of antibodies to lytic proteins by serologic studies.[79]

It is still not clear how EBV enters NPC or other epithelial cells. It enters B lymphocytes via the C3d/CD21 receptor, using the major envelope glycoprotein gp340 as a ligand.[80-82] Human epithelial cells express a surface protein that is antigenically related to the C3d receptor, containing an epitope that reacts with at least one CD21 antibody.[83] However, the epithelial surface protein has a different molecular weight than the lymphocyte C3d receptor and is expressed at low levels. Alternatively, EBV entry into epithelial cells may be mediated through other mechanisms such as via internalization of anti-EBV IgA antibodies.[84]

Several EBV proteins, most notably LMP-1, may have a significant effect on epithelial cells. LMP-1 is essential for EBV-mediated transformation of B lymphocytes into lymphoblastoid cell lines,[85] and has been shown to have oncogenic properties in transfected rodent fibroblast cell lines.[86] Transfection of epithelial cell lines with LMP-1 appears to inhibit differentiation.[87,88] For example, transfection of a keratinocyte cell line with the LMP-1 gene causes a striking morphologic transformation from flat, polygonal colonies to bundles of spindle-shaped cells that form multilayered foci, with down-regulation of keratin expression.[89] In addition, two other epithelial markers, an epithelial glycoprotein (EGP) recognized by the Ber-EP4 monoclonal antibody and epithelial membrane antigen (EMA), are also down-regulated by LMP-1,[88] although there appears to be no effect on variant CD44 expression.[90] LMP-1 also increases levels of surface expression of the membrane proteins CD40 and ICAM-1.[87] LMP-1 may act by signaling cell growth via TRAF3, a member of a family of tumor necrosis factor–associated factors (TRAFs) that also mediate CD40 cell activation.[91,92]

The amino terminus of the LMP-1 protein is essential to its actions, and deletion of this region abolishes its activity.[93] A characteristic 30 base pair deletion has been identified at the 3' end of the LMP gene, in the cytoplasmic domain, first identified in NPC.[94,95] Transfectants of these isolates have been found to be aggressive when inoculated into severe combined immunodeficiency (SCID) mice or nude mice, suggesting that the presence of this deletion may be associated with more aggressive behavior when found in tumors.[95,96] This deletion has been frequently detected in type A Chinese EBV strains, but has also been identified in a subset of cases of NPC from the Mediterranean and the United States, including cases with either type A or B EBV.[97]

CLINICAL FEATURES

Nasopharyngeal carcinoma most frequently arises in the fossa of Rosenmüller and sometimes in both fossae. Typically, it presents with a neck mass, nasal obstruction with epistaxis, and hearing loss or serous otitis media. But the tumor can present a variety of local, regional, and distant signs and symptoms (ie, head pain, facial pain or paresthesia, weight loss, diplopia, jugular foramen syndrome, etc). Identification of the earliest tumors is made possible by the high-quality optics available in both flexible and rigid endoscopes. The biopsy may be performed under either local or general anesthesia, but general anesthesia is rarely necessary for definitive biopsy of the nasopharynx.[98] Large biopsy forceps are essential to obtaining a representative tissue sample, and an accurate diagnosis can be obtained under direct vision.[98,99] Should the biopsy result not correlate with the clinical presentation, it may be repeated without delay under either local or general anesthesia.[98] Multiple random biopsies, including mucosal biopsies adjacent to Rosenmüller's fossa, are not justified in view of the high accuracy obtained with flexible and rigid endoscopes, which allow good visualization of the nasopharynx under local anesthesia and direct vision.[98] The most important factor in obtaining accurate diagnostic biopsies is the biopsy technique and not the type of anesthesia employed.[98] The sensitivity of this technique in diagnosing NPC was 95.1% under local anesthesia and 95.6% under general anesthesia.[98]

In a study by Lee et al,[100] only 9% of patients had disease confined to the nasopharynx with clinically absent lymph node metastasis. About 80% of patients with NPC have cervical lymph node metastases at the time of presentation, usually from an occult primary.[101-103] Many of the patients who are considered to have tumors limited to the nasopharynx may have retropharyngeal nodal involvement, which is actually the first level of nodes from this area.[104] External beam supervoltage irradiation is the mainstay of therapy for patients with NPC.[105] Among the different histologic subtypes of NPC, the keratinizing type has the poorest prognosis and is the least radiosensitive. Undifferentiated NPC, on the other hand, is radiosensitive, with a 60% to 70% survival rate compared to 20% for the keratinizing type of NPC of the same stage.[106,107] Recent studies have shown that UNPC is chemosensitive and that there is a potential curability when chemotherapy is used for recurrent or metastatic disease.[7,108]

The prognosis of patients with NPC is influenced by the extent of the primary tumor, the level of the disease in the neck, the histologic subtype of the tumor, the age of the patient, the number of symptoms, and the serum level of CD23, but also by the type and the technique of radiotherapy.[104,109] Patients with a primary tumor only have the best prognosis, and patients with small unilateral lymph nodes or base of skull destruction still have a relatively good survival.[3] However, the presence of large bilateral cervical lymph nodes or cranial nerve palsies has a major negative impact on survival. Survival rates are better in females and in the young.[15] The serum level of CD23 may represent an independent prognostic factor for initially nonmetastatic, locally advanced cases treated with chemotherapy and radiotherapy.[109]

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LETTERS TO THE EDITOR

To the Editor:

In a recent paper published in your journal, Shapiro et al (*Ann Otol Rhinol Laryngol* 1996;105:331-5) presented a series of seven patients with oropharyngeal dysphagia and inflammatory muscle involvement that they considered a new entity of myopathy. In their opinion, this entity is constituted by the association of the following: progressive solid food dysphagia, pharyngeal phase abnormalities (with pooling of secretions, decreased epiglottic deflection, and prominence of the cricopharyngeal muscle), normal neurologic findings, possible efficacy of cricopharyngeal (CP) myotomy, and pathologic characteristics consisting of foci of interstitial chronic inflammation, segmental necrosis, regenerating muscle fibers, and increase in connective tissue.

Only a few months earlier, we had published in the *Annals* an article describing identical histopathologic changes in the CP muscle with both a demonstration and critical discussion of such findings.[1] In our series of seven elderly patients complaining of oropharyngeal dysphagia and who had been improved by a CP myotomy, all of the CP muscles were pathologically examined and were characterized by an inflammatory infiltration, a decrease in size and number of the fibers, and an increase in connective tissue. We underlined that this could either be the consequence of a muscular process or have a neurogenic origin, both possibly related to aging. As we tried to point out in our article, there is absolutely no evidence that would enable one to describe these modifications as a "new entity" or as an "inflammatory" myopathy, and these are not even new findings, since they have already been described by other authors.[2,3]

We would like to reinforce the point that selective impairment of the pharyngeal stage of swallowing, whatever the upper esophageal function is, with decreased pharyngeal propulsion is not specific to a clinical entity or even of a group of diseases like myopathies. It has been previously described as a feature in the aged population or may be indicative of a neurologic disease, even though clinical and imaging investigations remain negative.

The normal appearance of the CP muscle has been extensively studied.[4,5] However, further investigation is required to improve our knowledge of the effect of aging on the swallowing process and the pharyngeal muscles.

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To the Editor:

We wish to thank Dr Jean Lacau St Guily and collaborators for their letter regarding our paper. Upon review of their letter, we would offer the following comments for possible clarification.

1. The report by Dr Lacau St Guily was published in the *Annals* a few months before ours, and consequently, was not available at the time of submission of the galleys for us to include in our reference citations. The illustrations of the histopathologic findings in Dr Lacau St Guily and coworkers' publication point to marked connective tissue infiltration; only the text describes inflammatory changes as well. Some of the changes described, though not illustrated, are similar to those we have reported; others are not at all similar.

2. The article by Cruse and collaborators cited by Dr Lacau St Guily and coworkers appears to us to emphasize atrophy of muscle fibers and connective tissue ingrowth (Figures 2-7). The authors specifically state that inflammation was not present in their cases, therefore suggesting that the entity they describe is different from the one that we described.

3. Dr Lacau St Guily and collaborators also cite another publication that supports their argument that the findings we described are not novel. (This is a citation in a monograph entitled *La bouche de l'oesophage*, by P. Mounier-Kuhn and collaborators.) Careful reading of this text and study of the published photomicrographs (Figures 27B and 27C) indicate that the findings described are those of diffuse interstitial fibrosis and connective tissue proliferation, without evidence of an inflammatory component in the illustrations or in the detailed description of the findings (page 70). Unaccountably, the interpretation of the lesion is that of "interstitial myositis" — an entity that is not well characterized in the literature.

It is always difficult to assign priority in clinical reports. We have tried to document with illustrations an entity that seems to us unique, though there is no telling if it has never been previously reported. As the saying goes, *En medicine c'est comme en amour: pas de jamais, pas de toujours.*

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To the Editor:

I welcome the article by Acquadro and Montgomery (*Ann Otol Rhinol Laryngol* 1996;105:607-14), "Treatment of Chronic Paranasal Sinus Pain with Minimal Sinus Disease."

The authors are to be commended for their meticulous presentation. The title of the article, however, points to a problem that plagues the practicing otolaryngologist. "Sinus pain" is a frequent complaint in the office, and the complaint tends to assume the conclusion. The concept of "sinus headache" is reinforced by Madison Avenue in its portrayal of the uses of various over-the-counter decongestants and pain medications. These assumptions have made our patients vulnerable to the notion that when sinus headaches are not cured by sinus headache medicine, they are then amenable to sinus surgery. The all-too-frequent failure to cure is attributed to inadequate or incomplete surgery, leading to revision surgeries. These patients frequently become understandably frustrated and bitter.

Few issues in otolaryngology would, from the viewpoint of our patients, benefit more from the rigorous approach taken by the authors. I only wish they had entitled the article "Head Pain." The distinction is by no means trivial.

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To the Editor:

The following is a response to the fine comment by Dr Harris. We wish to thank Dr Harris for his kind words, and we strongly agree with his observations and concerns. However, many patients are referred to otolaryngologists with a symptom described as "sinus pain" and a presumptive diagnosis of sinusitis. The otolaryngologist is in the best — though extremely difficult and sometimes impossible — position to determine if sinusitis exists. Even if no sinusitis exists, the nociceptive field area of pain (and sometimes referred area of pain) is in the paranasal sinus and other areas of the head, and can also be found in patients with sinusitis. An enlightened view will acknowledge that "sinus pain" should not assume a conclusion describing the neurophysiology and pathophysiology any better than "left arm pain" would assume a conclusion for a variety of possibilities, including complex regional pain type 1 or 2 (the newer taxonomy for reflex sympathetic dystrophy or causalgia), fracture of the humerus, tendinitis, cellulitis, nerve compression, or myocardial infarction without a proper history, physical examination, and diagnostic testing to support a diagnosis.

The term "head pain" was strongly considered in lieu of "paranasal sinus pain" for the very reason that Dr Harris correctly points out. Sinus pain should not assume a conclusion. However, we are speaking of pain that often appears to originate from the paranasal-parasinus nociceptive field, where many clinicians have difficulty determining a diagnosis. We should not be wary of describing a more precise perceived location of pain. A proper history, physical examination, and diagnostic testing are still necessary to determine the cause of "sinus pain," "head pain," or "headache." The field of medicine is still learning about the neurophysiology of pain, and continued efforts are needed to better define sinusitis.

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BOOK REVIEW

Auditory System Plasticity and Regeneration

Richard J. Salvi, Donald Henderson, Franco Fiorino, and Vittorio Colletti, editors. Hard cover, illustrated, indexed, 412 pages, 1996. Thieme Medical Publishers, New York, NY, $75.

This book is distinctive in that its precise orientations should stimulate discussions and new deductions concerning the pathophysiology of the ear. In the past, regeneration of damaged auditory hair cells was considered restricted to cold-blooded vertebrates, and damage to hair cells was considered irreversible. This book applies the results of current experimental investigations to higher organisms to evaluate the regenerative capacity of the hearing mechanism of humans. It does so by focusing on three areas of research: hair cell regeneration, "conditioning and toughening" of the peripheral auditory system, and plasticity and reorganization of the central auditory system.

Typically, certain growth factors are necessary to allow the organ of Corti to regenerate and replenish its hair cells and/or supporting cells. In regard to "toughening" of the auditory system, there are, it seems, molecular substances that prevent or reduce hearing losses from various causes, including sound trauma. Finally, and of great interest, if the central nervous system can reorganize subsequent to inner ear damage or destruction, can it reorganize following cochlear implantation? The third part of this book discusses these alterations.

This text contains an ingenious overview of experimental works. The information is thorough. The terminology is clearly presented. One does not need a postgraduate degree in biochemistry or molecular biology. Concepts of the hearing mechanism presented herein may well be the turning point of future therapy. The subject warrants the serious attention of physicians and those who specialize in conditions of the ear.

DENNIS G. PAPPAS, MD
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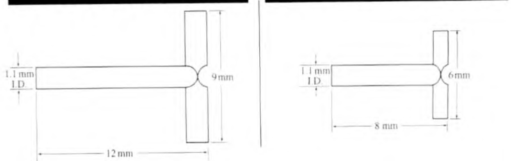

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SINGLE DOSE OF BETAMETHASONE IN COMBINED MEDICAL TREATMENT OF SECRETORY OTITIS MEDIA

CLAES HEMLIN, MD, PHD
CHRISTER CARENFELT, MD, PHD GEORGIOS PAPATZIAMOS, MD
STOCKHOLM, SWEDEN

With the objective of evaluating the efficacy of cefixime and the combination of cefixime and betamethasone in the treatment of secretory otitis media (SOM), we enrolled 142 children 2 to 12 years old in a randomized, double-blind, placebo-controlled study. All children suffered from SOM, verified by otomicroscopy and tympanometry, of at least 3 months' duration. Active treatment was a 10-day course of cefixime with and without a single dose of 6 mg betamethasone. One hundred forty children were available for efficacy evaluation 14 to 23 days after the start of treatment. A statistically significant treatment effect was found in the group treated with cefixime plus betamethasone ($n = 59$), with a 44.1% cure rate as compared to 19.7% in the cefixime-treated group ($n = 61$; $p < .005$) and 5% in the placebo group ($n = 20$; $p < .005$). Relapse rates were high and no statistically significant differences between groups remained at last valid visit. Adverse events possibly or probably associated with active treatment were reported in 11 cases (9.2%). One patient reported a severe adverse event. No serious events occurred. The study did not show any significant long-term effect of cefixime treatment or any long-term treatment benefit with the addition of betamethasone to the antibiotic.

KEY WORDS — betamethasone, cephalosporin, secretory otitis media.

INTRODUCTION

Few disorders of children are as prevalent and yet as therapeutically frustrating as secretory otitis media (SOM). Despite years of worldwide research efforts, the cause still remains much of an enigma. Numerous studies have shown that the middle ear effusion (MEE) represents an inflammatory reaction. It is therefore natural that anti-inflammatory drugs such as corticosteroids have been used in therapeutic trials, either alone or in combination with antibiotic drugs. In a recently performed meta-analysis,[1] 12 such studies were found, from which 6 were selected for a meta-analysis based on quality criteria.[2-7] The pooled results produced an odds ratio of 3.0 in favor of steroid over placebo treatment. Not included in the meta-analysis were two randomized, double-blind studies[8,9] both showing statistically significant improvement in the groups treated with a combination of antibiotics and corticosteroids.

Although long considered a noninfectious disease, SOM has been found to be associated with a heavier load of potentially pathogenic bacteria in the nasopharynx than is found in healthy children.[10] The assumption that the MEE is sterile has been revised in recent decades. The finding of growing pathogens in at least 20% of MEEs and an overall finding of bacteria via microscope in up to 70% of MEEs have been documented.[11] Nevertheless, treatment effects of orally administered antibiotics are limited, as found in two recently published meta-analyses.[12,13] The increase in short-term pooled cure rate due to antibiotic treatment was statistically significant in both studies and estimated to be 22%[12] and 16%,[13] respectively, of the patients treated, whereas no statistically significant long-term effect could be found.[13] Antibiotics used in these studies included erythromycin, cefaclor, amoxicillin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, and erythromycin-sulfisoxazole.

We have conducted a randomized, placebo-controlled study among children with defined SOM to examine whether antibiotic treatment is effective in eliminating MEE and whether the efficacy of antibiotic treatment is strengthened by a single dose of corticosteroid. Following the inhibition of bacterial growth and the reduction of the antigenic or toxic burden of the middle ear pathogens, the steroid dose is hypothesized to moderate the tubal inflammation, improve tubal function, and facilitate the aeration of the middle ear.

Cefixime, a third-generation oral cephalosporin, was chosen to achieve a broad antibacterial treatment effect, including β-lactamase–producing organisms. Betamethasone was administered in a dosage com-

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From the Department of Otorhinolaryngology, Karolinska Hospital, Stockholm, Sweden. ASTRA AB supplied the drugs and patient registration forms, and assisted in the data analysis.

Presented in part at the Second Extraordinary International Symposium on Recent Advances in Otitis Media, Oita, Japan, March 31–April 3, 1993. Part of thesis (C.H.), Karolinska Institute, 1994.

CORRESPONDENCE — Claes Hemlin, MD, PhD, Dept of Otorhinolaryngology, Karolinska Hospital, S-171 76 Stockholm, Sweden.

monly used in children with subglottic laryngitis.

MATERIAL AND METHODS

The trial was carried out at the otolaryngology department of Karolinska Hospital in Sweden, and 142 children of both sexes, 2 to 12 years of age, were included in the study.

The inclusion criterion was unilateral or bilateral SOM of at least 3 months' duration as confirmed by otomicroscopy and tympanometry. An immobile and pale eardrum when examined with otomicroscopy at a visit at least 3 months prior to the start of the study treatment should have been found. At the time of inclusion in the study, the same otomicroscopic finding as well as a tympanogram showing a flat, Jerger type B curve in at least one of the ears had to be found. All participating children were treated as outpatients.

Children were excluded from entry if any of the following criteria applied to them: a severe underlying disease, immunologic deficiency, cleft palate, a known or suspected allergy to penicillins or cephalosporins, a history of an antibacterial treatment within the preceding 4 weeks, or previous inclusion in the study.

Upon inclusion, a detailed patient history was obtained concerning previous ear diseases, and the presence of hearing loss, chronic nasal obstruction, chronic nasal secretion, and nasal sniffing. A nasopharyngeal specimen for bacterial culture was obtained.

Treatment with cefixime or cefixime plus betamethasone or placebo was allocated at random with a ratio of 3:3:1 (cefixime to cefixime plus betamethasone to placebo). The drugs were dispensed double-blind by a double-dummy technique. A liquid suspension of cefixime (20 mg/mL) was administered in a dosage of 8 mg/kg body weight per day in morning and evening doses for 10 consecutive days. The betamethasone tablets were given in a single dose of 6 mg on the morning of day 10. A placebo suspension and tablets with a similar appearance were used. Antimicrobial agents other than the study drugs were not allowed during the study period. Any other medication considered necessary for the patient's welfare was allowed.

Two to 11 days after completion of the initial treatment, the children were reexamined with otomicroscopy and tympanometry (visit 2). Children showing a normal middle ear status in at least one ear (if bilateral SOM at the time of inclusion) or both ears (if unilateral SOM at inclusion) were classified as cured. The remaining children were classified as treatment failures and were not followed further in this study. A normal middle ear status was defined as the otomicroscopic finding of a pale eardrum with normal mobility and a tympanogram showing a type A curve or a type C curve with a peak of more than –300 decapascals.

During the same visit, compliance was ensured by the return of packages subsequently measured for the remaining suspension and the return of a home record form for registering the daily intake. Adverse events that occurred were also registered and classified by type, intensity, duration, and other possible explanations for the occurrence. Intensity definitions were as follows: mild (does not hinder the pretreatment functioning but is mildly irritating), moderate (discomfort is enough to cause interference with pretreatment functioning), severe (significant impairment of pretreatment functioning or incapacitation), and serious (constitutes a definite hazard or handicap to the patient).

The patients who were found to be cured at this follow-up visit were scheduled for two more visits, after 6 weeks and 6 months, respectively. The otomicroscopy and the tympanometry were repeated, and the same criteria for cure were used as at visit 2.

Withdrawal from the study treatment due to adverse events or any other reason was recorded in the case record form with details about the treatment duration and the specific reason for withdrawal.

The bacteriologic samples from the nasopharynx at inclusion and at visit 2 were cultured and analyzed for aerobic bacteria. Susceptibility tests were performed against cefixime, cefaclor, cephalexin, penicillin V, and ampicillin by means of the disc diffusion method, and the susceptibility was interpreted according to the S-I-R (sensitive-intermediary-resistant) system, as used in Sweden.[14] *Haemophilus influenzae* and *Moraxella catarrhalis* were screened for β-lactamase activity by the nitrocefin test.

The main efficacy variable was the healing rate at visit 2. Assuming a placebo healing rate of 15%, a cefixime healing rate of 50%, and a cefixime-betamethasone healing rate of 75%, a total of 140 evaluable patients (20 + 60 + 60) are needed with a 1:3:3 randomization. This is based on pairwise χ^2 tests on the level $\alpha = 5\%$ and a power of 80% in each pairwise test.

The healing rates at the first follow-up visit were compared by means of pairwise χ^2 tests. The same method was used to compare relapse rates at later visits and the proportion of patients who reported adverse events. Student's *t*-test, χ^2, and Mann-Whitney U were used to compare historical and bacteriologic data between healed and nonhealed children in

TABLE 1. PATIENT CHARACTERISTICS

	Cefixime + *Betamethasone*	*Cefixime* + *Placebo*	*Placebo* + *Placebo*
Number	59	61	20
Age (mean, mo)	67	63	63
Males/females	36/23	37/24	14/6
Unilateral/bilateral SOM	9/50	9/52	2/18
Months of inclusion			
Dec-Feb	14	12	6
March-May	30	30	9
June-Aug	4	2	1
Sept-Nov	11	17	4
AOM episodes*			
0	32	30	13
1	11	14	7
2	6	10	0
>2	10	7	0
TMD procedures†			
0	36	44	14
1	11	7	4
2	7	4	2
>2	5	6	0

SOM — secretory otitis media.

*Number of acute otitis media (AOM) episodes during 12 months preceding inclusion into study according to patient history.

†Number of transmyringeal drainage (TMD) procedures during earlier life according to patient history.

the cefixime-betamethasone group.

The study was performed in accordance with the principles of the Declaration of Helsinki. The protocol and the written patient information were reviewed by the ethics committee of the Karolinska Institute prior to the start of the study. Oral consent was obtained from the parents.

RESULTS

Of 142 patients admitted to the study, 140 remained valid for efficacy analysis. One patient, belonging to the cefixime-treated group, discontinued treatment because of an adverse event. Another patient, randomized to the cefixime plus betamethasone group, withdrew from the study before starting treatment. Only minor differences in baseline data were found between the treatment groups (Table 1). There were no significant differences in the nasopharyngeal bacterial flora between the groups. *Haemophilus influenzae* was isolated from 61%, *Streptococcus pneumoniae* from 48%, and *M catarrhalis* from 49% of children cultured. At least one of these species was isolated from 87% of these children.

TABLE 2. TREATMENT RESULTS AT VISIT 2, 14 TO 23 DAYS AFTER TREATMENT START

	Cefixime + *Betamethasone* *(n = 59)*	*Cefixime* + *Placebo* *(n = 61)*	*Placebo* + *Placebo* *(n = 20)*	*Total* *(n = 140)*
Cured	26 (44.1%)	12 (19.7%)	1 (5.0%)	39 (27.9%)
Failure	33 (55.9%)	49 (80.3%)	19 (95.0%)	101 (72.1%)

Cefixime versus cefixime plus betamethasone, $p < .005$. Placebo versus cefixime plus betamethasone, $p < .005$. Cefixime versus placebo, not significant.

Of the 140 treated patients, 39 were considered cured at visit 2. Of these, 26 had been treated with cefixime plus betamethasone, giving this treatment group a significantly better treatment effect compared to both other groups (Table 2). Significantly better treatment effects for the cefixime plus betamethasone group were also found in analyzing treatment effects for the 260 ears treated. The healing rates for ears were 37% in the cefixime plus betamethasone group, 14% in the cefixime plus placebo group, and 3% in the placebo plus placebo group. Similar significant differences were found when analyzing only children with bilateral SOM at inclusion. Children with unilateral SOM could not be analyzed separately due to too-small groups (data not shown).

The recurrence rate was high, and at the following visits, no statistically significant differences remained between the groups (Table 3). In comparing the fraction of healed to nonhealed patients in the cefixime plus betamethasone group, no significant differences were found regarding age, laterality of disease at inclusion, season of inclusion, history of acute otitis media, transmyringeal drainage treatment, nasal secretion, sniffing, or carriage of nasopharyngeal pathogens. However, a statistically significant difference was found regarding history of nasal obstruction, with the presence of such history indicating a lower probability of healing (Table 4). A nonsignificant increase in the load of potential pathogens in the nasopharynx was found in the group healing after treatment (Table 4).

Unilateral complete resolution of MEE was con-

TABLE 3. LONG-TERM TREATMENT EFFECT

	Cefixime + *Betamethasone*	*Cefixime* + *Placebo*	*Placebo* + *Placebo*
Healed at 2 wk	26 (44.1)	12 (19.7)	1 (5.0)
Lost to follow-up at 6 wk	3	1	0
Relapsed within 6 wk	15	3	1
Healed at 6 wk	8 (13.6)	8 (13.1)	0
Lost to follow-up at 6 mo	1	0	0
Relapsed within 6 mo	0	1	0
Healed at 6 mo	7 (11.9)	7 (11.5)	0

Figures in parentheses are percentages of number of patients in group at inclusion.

TABLE 4. COMPARISON OF BASELINE DATA BETWEEN HEALED AND NONHEALED PATIENTS IN CEFIXIME PLUS BETAMETHASONE GROUP

	Healed at 2 wk (n = 26)	*Not Healed at 2 wk (n = 33)*	*Statistical Analysis*
Age (mo)	65	69	NS *t*
Unilateral SOM	12%	18%	NS χ^2
Months of inclusion			
Dec-Feb	8	6	
March-May	12	18	
June-Aug	1	3	
Sept-Nov	5	6	NS χ^2
History of			
AOM episodes (mean)*	1.0	1.2	NS U
TMD procedures (mean)†	0.8	0.8	NS U
Hearing loss	88%	97%	NS χ^2
Nasal obstruction	35%	64%	$p < .05$ χ^2
Nasal secretion	8%	21%	NS χ^2
Sniffing	19%	35%	NS χ^2
Number with nasopharyngeal cultures	23	28	
Any pathogen	100%	88%	NS χ^2
Haemophilus influenzae	65%	50%	NS χ^2
Streptococcus pneumoniae	57%	50%	NS χ^2
Moraxella catarrhalis	65%	50%	NS χ^2
β-Lactamase–producing pathogens	17%	37%	NS χ^2
Pathogen species/specimen	2.04	1.57	NS U

NS — no statistically significant difference between healed and nonhealed groups of children, *t* — Student's *t*-test, U — Mann-Whitney U.

*Number of acute otitis media episodes during 12 months preceding inclusion into study according to patient history.

†Number of transmyringeal drainage procedures during earlier life according to patient history.

TABLE 5. ADVERSE EVENTS CLASSIFIED AS PROBABLY OR POSSIBLY RELATED TO ACTIVE TREATMENT

	Cefixime + Betamethasone (n = 10)	*Cefixime + Placebo (n = 6)*
Dermatitis	1 (1.7%)	0 (0.0%)
Diarrhea	4 (6.7%)	2 (3.3%)
Loose stools	2 (3.3%)	2 (3.3%)
Vomiting	1 (1.7%)	3 (4.9%)
Stomach pain	2 (3.3%)	2 (3.3%)
Gastroenteritis	0 (0.0%)	1 (1.7%)

Data are numbers of children reporting adverse event. Percentages are of study group. One child could report more than one symptom. These adverse events did not occur in placebo plus placebo group.

sidered sufficient for healing. In the cefixime plus betamethasone group, 23 of 26 healed patients had bilateral SOM at the time of entry. At visit 2, 12 had unilateral SOM, and the eardrums in the remaining 14 were bilaterally normal. Of the 7 patients classified as healed at 6-month control, 3 were unilaterally and 4 were bilaterally healed.

A total of 24 children were reported as experiencing adverse events. For 16 of these children the adverse events were classified as possibly or probably treatment-related. Symptoms reported by the 16 children are shown in Table 5. Of the symptoms reported, 65% were considered mild and the remainder moderate. One child reported fever and sore throat that was classified as a severe adverse event, but considered to have an unlikely association with treatment given. None of the adverse events were classified as serious.

DISCUSSION

The purpose of this study was to evaluate whether surgery could be avoided in a significant number of patients with SOM by treating them with cefixime plus betamethasone or cefixime alone. Entry criteria therefore included well-defined SOM of at least 3 months' duration verified by both otomicroscopy and tympanometry. The outcome criterion was a complete healing of at least one ear making it possible to postpone surgery. As a consequence, the design of this study does not allow for any overestimation, but does risk some underestimation of treatment effect, since patients who improved but who were not healed after treatment were classified as treatment failures at visit 2.

The results of the study show a statistically significant short-term effect of the combined treatment, but a high recurrence rate. The long-term effect of cefixime treatment is the same regardless of whether betamethasone is used or not, and there seems to be a favorable long-term effect for about 13% of the patients.

The results of this study further support the theory that corticosteroids have a significant effect in combination with antibiotics.[1] Furthermore, they show that a single dose of betamethasone given at the end of antibiotic treatment has a short-term effect comparable to that obtained with 7 to 14 days' treatment in studies using antibiotics and steroids.[4-6] Since the trial did not include any group solely receiving betamethasone treatment, no conclusions regarding the effect of a single dose of betamethasone can be drawn.

Cefixime treatment alone did not show any statistically significant treatment effect compared to placebo treatment, although there was a 15% rate difference in the cefixime-treated group. A treatment effect of a similar level was reported in the two meta-analyses.[12,13] In both these studies the differences were statistically significant. Thus, the 15% rate difference in the cefixime group may well reflect a true treatment effect. The failure to reach a statistically significant difference is most likely due to a type II error. The study design, which did not identify partial improvements, may also have led to an underestimation of treatment effects. This study also lacks sufficient power to verify the apparent long-term effect of cefixime treatment.

Making conclusions with regard to the role of antibiotic treatment of SOM in general is also hampered by the fact that cefixime has been found not to have the same broad antibacterial activity clinically as in vitro.[15] In particular, poor activity against *S pneumoniae* has been found.[16] Another antibiotic might have yielded a better result.

When determining the role of antibiotic treatment of SOM, not only must therapeutic efficacy be considered, but also ecologic factors. The development of resistant upper airway pathogens, and especially multi–drug-resistant *S pneumoniae*,[17] is a serious threat to the community, and therefore restrictiveness is warranted in antibiotic treatment recommendations.

The association between healing after treatment with cefixime plus betamethasone and a low frequency of nasal obstruction history and a tendency for an increased bacterial load in the nasopharynx is interesting, but needs further study, possibly leading to the development of better methods for the identification of which patients will benefit from antibiotic with or without corticosteroid treatment.

The results of this study support the theory that one important etiologic factor in chronic SOM in children is an inflammatory process caused by pathogenic bacteria, that this process can be influenced by anti-inflammatory and antibiotic drugs, and that there is therefore a rationale for including such drugs in the therapeutic arsenal. The exact role of a combination of cefixime and betamethasone in treatment needs to be further elucidated, with a special emphasis on identifying those patients for whom the treatment is beneficial. The results of this study do not permit a general recommendation of the use of such treatment.

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CRICOHYOIDOEPIGLOTTOPEXY FOR GLOTTIC CARCINOMA WITH FIXATION OR IMPAIRED MOTION OF THE TRUE VOCAL CORD: 5-YEAR ONCOLOGIC RESULTS WITH 112 PATIENTS

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The medical charts and operative files of 112 patients (combined inception cohort) with well to moderately differentiated invasive glottic squamous cell carcinoma presenting fixation (22) or impaired motion (90) of the true vocal cord (TVC) consecutively treated with cricohyoidoepiglottopexy (CHEP) at our institutions from 1972 to 1989 were retrospectively reviewed. A minimum 5-year follow-up was always achieved. The Kaplan-Meier 5-year actuarial survival, local recurrence, nodal recurrence, distant metastasis, and metachronous second primary tumor estimate for the entire group of patients were 84.7%, 5.4%, 6.4%, 1.2%, and 10.8%, respectively. The 5-year absolute and cause-specific survival rates were 85.5% and 94.1% for patients with fixation of the TVC and 81.3% and 96% for patients with impaired motion of the TVC. The 5-year actuarial local control rates for patients with fixation or impaired motion of the TVC were 95.4% and 94.4%, respectively. Local recurrence was statistically more likely in patients with positive margins ($p = .007$). Nodal recurrence was statistically more likely in patients with local recurrence ($p = .0005$). Permanent tracheostomy related to postoperative laryngeal stenosis was requested in 2 patients. Aspiration-related completion total laryngectomy and/or permanent gastrostomy were never requested. Overall, local control and laryngeal preservation were achieved in 97.3%, and 95.5% of patients, respectively. At our institutions, the change from the conservative treatment modalities of radiotherapy and vertical partial laryngectomy to CHEP has brought about an increase in long-term survival, local control, and laryngeal preservation rates when compared to historical controls using vertical partial laryngectomy or radiotherapy.

KEY WORDS — cricohyoidoepiglottopexy, glottic squamous cell carcinoma, partial laryngeal surgery.

INTRODUCTION

Vertical partial laryngectomy (VPL) and radiotherapy (RT) are the conservative treatment modalities advocated for glottic squamous cell carcinoma with impaired motion or fixation of the true vocal cord (TVC). However, in series with more than 20 patients and a minimal 2-year follow-up, local recurrence is reported to be 27% to 49% in patients with impaired TVC motion and 11% to 50% in patients with fixation of the TVC (Table 1[1-16]). Hendrickson[17] furthermore reported that only about 50% of those patients with T2 glottic tumors who failed following initial RT were salvaged by subsequent surgery. Viani et al,[18] in a recent analysis of recurrence after RT for T1-3N0 glottic carcinoma, also underscored the adverse prognosis of local recurrence. In their series, 13% of patients with a recurrent primary tumor were untreatable, the 5-year survival rate after a primary tumor recurrence was 39%, the mortality of patients in the hospital after salvage laryngectomy was 3%, and 30% of patients undergoing a salvage total laryngectomy had a pharyngocutaneous fistula.[18]

In the 1970s a new concept in partial laryngeal surgery for glottic carcinoma, the cricohyoidoepiglottopexy (CHEP) procedure, was introduced into our departments following the initial reports of Majer and Rieder[19] and Piquet et al,[20] in an attempt to increase long-term local control and laryngeal preservation for glottic carcinoma with fixation or impaired motion of the TVC.

The preservation of at least one mobile cricoarytenoid unit together with the impaction between the cricoid cartilage, hyoid bone, and remaining epiglottis resulted in preservation of laryngeal structures that support voicing, swallowing, and respiration without the need for a permanent tracheostomy. Objective measurements of speech and voice after CHEP noted that speech and voice parameters were

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TABLE 1. REPORTED LOCAL RECURRENCE RATE AFTER RT OR VPL FOR GLOTTIC CARCINOMA WITH IMPAIRED MOTION OR FIXATION OF TRUE VOCAL CORD IN SERIES WITH MORE THAN 20 PATIENTS

Authors	*Year*	*Treatment Modality*	*No. of Pts*	*Local Failure (%)*	*Follow-up (y)*
Impaired motion of true vocal cord					
Harwood and De Boer[1]	1980	RT	52	49	NA
Wang[2]	1986	RT	88	39	5
Mendenhall et al[3]	1988	RT	43	28	2
Howell-Burke et al[4]	1990	RT	73	27	NA
Wiggenraad et al[5]	1990	RT	23	29	2
Laccourreye et al[6]	1991	VPL	31	32	3
Turesson et al[7]	1991	RT	53	30	4
Inoue et al[8]	1992	RT	34	37	5
Fixation of true vocal cord					
Kirchner and Som[9]	1971	VPL	22	41	NA
Stewart et al[10]	1975	RT	67	43	10
Som[11]	1979	VPL	26	42	NA
Harwood et al[12]	1980	RT	112	49	5
Skolyszewski and Reinfuss[13]	1981	RT	91	50	3
Kessler et al[14]	1987	VPL	27	11	2
Robson et al[15]	1990	RT	39	33	5
Parsons et al[16]	1993	RT*	28	32	2

RT — radiotherapy, VPL — vertical partial laryngectomy, NA — not available.

*Twice a day.

comparable with the parameters recorded in normal adult laryngeal speakers, demonstrating thus that this surgical procedure should be viewed as a true conservative laryngeal procedure.[21]

This retrospective study was designed to document the long-term oncologic results achieved with CHEP in patients with invasive squamous cell carcinoma of the glottis presenting fixation or impaired motion of the TVC. Our study focuses upon 5-year actuarial survival, local recurrence, and nodal recurrence. Furthermore, the following independent variables — sex, age, exact tumor location, impaired motion or fixation of the TVC, T-stage, neoadjuvant chemotherapy, positive margins of resection, and thyroid cartilage invasion — were tested for potential correlation with survival, local recurrence, and nodal recurrence.

TABLE 2. CLINICAL AND PATHOLOGIC DATA

	Patients (n = 112)	
Clinical and Pathologic Features	*No.*	*%*
True vocal cord motion		
Impaired	90	80.4
Fixed	22	19.6
Specific site involved		
Anterior commissure	68	60.7
Contralateral true vocal cord	19	17.0
Floor of ventricle	72	64.3
Petiole of epiglottis	3	2.7
False vocal cord	6	5.4
Anterior aspect of arytenoid	11	9.8
Anterior subglottis	5	4.5
Lateral subglottis	12	10.7
T-stage		
T2	90	80.4
T3	22	19.6
Neoadjuvant chemotherapy	29	25.9
Margin of resection		
Negative margins	109	97.3
Positive margins	3	2.7

MATERIALS AND METHODS

One hundred twelve patients with previously untreated well to moderately differentiated invasive squamous cell carcinoma of the glottis with fixation or impaired motion of the TVC, without palpable cervical lymph nodes or synchronous second primary tumors, treated with CHEP between 1972 and 1989, took part in this retrospective study. Eighty-three patients were treated at Huriez Hospital (University Lille II) and 29 at Laënnec Hospital (University Paris V). There were 110 men and 2 women with ages ranging from 29 to 80 years (median age 55 years).

Details of tumor extent are summarized in Table 2. All patients had preoperative panendoscopy with 30° and 70° rigid telescopes. The infraglottic extent of the tumor never exceeded 10 mm anteriorly and 5 mm laterally. The TVC motion and arytenoid cartilage motion were separately analyzed at the time of indirect laryngoscopy. Videostroboscopy was not sys-

TABLE 3. UNIVARIATE ANALYSIS OF LOCAL RECURRENCE, NODAL RECURRENCE, AND SURVIVAL

	p Values		
Independent Factors	*Local Recurrence*	*Nodal Recurrence*	*Survival*
Age	.6	.9	.0502
T-stage	.66	.69	.8
Site involved			
Anterior commissure	.9	.6	.7
Floor of ventricle	.1	.5	.1
False vocal cord	.9	.9	.3
Anterior aspect of arytenoid	.8	.3	.9
Petiole of epiglottis	.9	.9	.8
Anterior subglottis	.7	.9	.1
Lateral subglottis	.9	.7	.5
Neoadjuvant chemotherapy	.5	.2	.6
Positive margins	.007	.02	.8
Thyroid cartilage invasion	.9	.9	.6
Local recurrence		.0005	.2
Nodal recurrence			.8

tematically performed. The arytenoid cartilage was never fixed. Computed tomography was performed after 1987, and in the early years patients had laryngograms. Preepiglottic space invasion was not encountered. Staging (Table 2) was according to the 1987 American Joint Committee on Cancer staging classification system.[22]

The induction chemotherapy regimen, performed in patients managed at Laënnec Hospital, consisted of cisplatin (20 mg/m^2 per day) and fluorouracil (1 mg/m^2 per day) administered as daily continuous intravenous dosages. The total number of treatment courses was determined according to the clinical response and the toxicity. Three courses were performed on average (range 2 to 5). The course duration was 4 days during the early years and 6 days since 1988. Induction chemotherapy was administered with 15 to 21 days' hiatus between courses. The reasons for this management policy have previously been reported and will not be detailed in the present report.[23] In the present study, surgery was not appreciably influenced by the response to chemotherapy.

The CHEP technique will not be described, since it was previously reported in the North American literature.[24,25] Our attitude toward the arytenoid cartilage on the tumor-bearing side varied according to the data of the laryngeal computed tomography, and the intraoperative assessment of the exact tumor extent. The arytenoid cartilage was totally resected in 90 patients. In 8 patients, the arytenoid cartilage was transected. In 14 patients both arytenoid cartilages were spared. An ipsilateral modified functional neck dissection, taking nodes at levels II-V and sparing cranial nerve XI and the internal jugular vein, was associated in 42 patients.

Postoperative RT, due to nodal invasion, was performed in 3 patients. The RT delivered 45 Gy to the neck and remaining larynx. Pathologic analysis of the specimen noted positive margins and thyroid cartilage invasion in 3 and 5 patients, respectively (Table 2).

Arytenoid cartilage fixation, an infraglottic extent of tumor reaching the upper border of the cricoid cartilage, and cricoid cartilage invasion were not present in our series, as such features are considered at both departments as major contraindications for CHEP completion.[24,25] Preepiglottic space invasion was never encountered, as this feature, in our opinion, should lead to completion cricohyoidopexy.

Follow-up data were collected via periodic visits to our departments. All patients were followed up for 5 years or until death. Statistical analysis of survival, local control, nodal control, and survival (absolute, and corrected from intercurrent disease and metachronous second primary tumor) incidence was based on the product limit method of Kaplan and Meier.[26] Univariate analysis of the correlation of the following independent factors — sex, age, exact tumor location, impaired motion or fixation of the TVC, T-stage, neoadjuvant chemotherapy, positive margins of resection, and thyroid cartilage invasion — to survival, local control, and nodal control was performed. The parametric χ^2 test and the nonparametric Mann-Whitney U test were used for qualitative and quantitative variables analyses, respectively.

RESULTS

No patient died in the postoperative period. The 5-

year absolute and cause-specific actuarial survival rates were 84.7% and 95.6%, respectively. The causes of death were metachronous second primary tumor, intercurrent disease, local recurrence, associated nodal and local recurrence, and distant metastasis in 10, 5, 1, 1, and 1 patients, respectively. The 5-year absolute and cause-specific survival rates for patients with fixation of the TVC were 85.5% and 94.1%, respectively. The 5-year absolute and cause-specific survival rates for patients with impaired motion of the TVC were 81.3% and 96%, respectively. No statistical relationship was noted between the variables under analysis and survival (Table 3).

Six patients had a local laryngeal recurrence, resulting in a 5-year actuarial local control rate of 94.6%. The 5-year actuarial local control rates for patients with fixation or impaired motion of the TVC were 95.4% and 94.4%, respectively. In univariate analysis (Table 3) local recurrence was statistically more likely if the margins of resection were positive ($p = .007$). Salvage therapy was total laryngectomy, total laryngectomy with postoperative RT, RT, and carbon dioxide laser resection in 2, 2, 1, and 1 patients, respectively. Permanent tracheostomy, related to postoperative laryngeal stenosis, was requested in 2 patients. Aspiration-related completion total laryngectomy and/or permanent gastrostomy were never requested. Overall, local control and laryngeal preservation were achieved in 97.3% (109/112) and 95.5% (107/112) of patients.

Recurrence in the neck, noted in 9 patients, resulted in a 5-year actuarial nodal control rate of 93.6%. In univariate analysis (Table 3) nodal recurrence was statistically more likely if the margins of resection were positive ($p = .02$) or if there was local recurrence ($p = .0005$).

One patient had distant metastasis involving lung, brain, and bone, resulting in a 5-year actuarial distant metastasis rate of 1.2%. The 5-year actuarial metachronous second primary tumor rate was 10.8%. Metachronous second primary tumors involved the lung, esophagus, hypopharynx, oropharynx, liver, and prostate in 5, 3, 2, 1, 1, and 1 patients, respectively.

DISCUSSION

Anatomicopathologic studies of serially sectioned laryngeal surgical specimens have revealed that fixation of the TVC in glottic carcinoma resulted from paraglottic space invasion with extensive involvement of the thyroarytenoid muscle.[9,27,28] Hirano et al[28] recently reported that invasion of one fourth to three fourths of the thyroarytenoid muscle resulted in impaired motion, while invasion of more than three fourths of the thyroarytenoid muscle caused fixation of the TVC. One of the main oncologic and surgical concepts supporting the CHEP procedure is the wide and complete removal of the thyroarytenoid muscle at the time of resection.[24,25] The CHEP procedure furthermore allows for complete resection of the lateral and posterior cricoarytenoid muscles if arytenoid cartilage disarticulation on the tumor-bearing side is performed.[24,25] In recent years, various reports in the North American literature have discussed the oncologic value and limitations of the CHEP procedure for glottic carcinoma.[23-25] However, none of these reports was specifically devoted to glottic carcinoma with fixation or impaired motion of the TVC, or presented a large series of patients with more than a 3-year follow-up.

Retrospective analysis of the medical charts of patients with glottic carcinoma consecutively treated with CHEP at our institutions from 1972 to 1989 revealed 112 patients with fixation or impaired motion of the TVC on the tumor-bearing side. Six patients in our series had a local laryngeal recurrence, resulting in a 5-year actuarial local control rate of 94.6%. The 5-year actuarial local control rates for patients with fixation and impaired motion of the TVC were 95.5% and 94.4%, respectively. If we compare such results with the reported local control rate in series with more than 20 patients and a minimum 2-year follow-up managed with VPL or RT (Table 1), we note that local recurrence drops from 27% to 49% down to 5.6% if the motion of the TVC is impaired and from 11% to 50% down to 4.5% if the TVC is fixed.[1-16] The wide resection of the paraglottic space and thyroid cartilage achieved with CHEP, and the completion of an arytenoid cartilage disarticulation on the tumor-bearing side in 80.4% of patients (90/112), together with the respect of the major contraindications for CHEP, explain, in our opinion, the high rate of local control achieved in our series. The process of selecting T3 glottic carcinomas amenable to CHEP involves careful analysis of data obtained from indirect laryngoscopy, direct laryngoscopy with 30° and 70° optics, laryngeal computed tomography, and direct tumor inspection at the time of surgery. Fixation of the arytenoid cartilage, cricoid cartilage invasion, preepiglottic space invasion, infraglottic extent of tumor reaching the upper border of the cricoid cartilage, tumor extent across the posterior commissure to involve the contralateral arytenoid cartilage, and extralaryngeal spread of tumor should be viewed as major contraindications to CHEP completion.[24,25] Salvage treatment in our series allowed for an overall 97.3% 5-year local control rate and a 95.5% 5-year laryngeal preservation rate. Soo et al,[29] in a multivariate analysis of 175 patients treated with VPL, reported that positive margins were a significant covariate affecting local recurrence. Univariate analysis in our series demonstrated that positive margins were the only significant covariate affecting local recurrence after CHEP (Table 3, p = .007). Nodal recurrence, in our series, was observed in 9 patients, resulting in a 5-year actuarial nodal control rate of 93.6%.

Univariate analysis (Table 3) revealed that local recurrence (p = .0005) and positive margins of resection (p = .02) were the only statistical predictors for nodal recurrence. Johnson et al,[30] in an analysis of open surgical therapy for glottic carcinoma, reported that elective neck dissection resulted in the identification of nodal metastasis in 10.4% of patients with T3 lesions who initially had no palpable evidence of cervical metastasis. All patients in our series were classified as N0. The completion of an ipsilateral modified functional neck dissection at the time of surgery in 42 patients, in our series, allowed for detection and treatment of nodal invasion in 2 patients, and of intranodal fibrosis in 2 other patients treated with neoadjuvant chemotherapy. Our results suggest, therefore, that the key point for nodal control in patients with glottic carcinoma presenting fixation or impaired motion of the TVC is the achievement of local control at primary treatment. However, in patients with glottic carcinoma presenting fixation of the TVC, we still advocate the completion of an ipsilateral modified neck dissection at the time of CHEP.

Achievement of a high local control rate in patients with glottic tumors presenting fixation or impaired motion of the TVC appeared to be valid in terms of survival. In our series, the 5-year absolute and cause-specific survival rates were 85.5% and 94.1% for patients with fixation of the TVC, and 81.3% and 96% for patients with impaired motion of the TVC. The reported 5-year absolute and cause-specific survival rates for glottic tumors with fixation of the TVC treated with RT vary from 41% to 59% and from 60% to 80%, respectively.[12,15,31] Harwood and De Boer[1] also reported a 75.2% 5-year corrected survival for glottic tumor with impaired motion of the TVC treated with RT. Metachronous second primary tumor and intercurrent disease were by far (10 and 5 patients, respectively) the main causes of death in our series.

None of the variables under analysis in our series were statistically related to survival (Table 3). At first glance, this might lead to the conclusion that induction chemotherapy is useless in patients with glottic carcinoma presenting fixation or impaired motion of the TVC. This assumption, in our opinion, is correct if the definitive treatment following induction chemotherapy is surgical resection with CHEP, including arytenoid cartilage disarticulation on the tumor-bearing side. However, as already reported by the Laënnec group,[32-34] induction chemotherapy allowed for a complete histologic response in 37.3% of patients with glottic carcinoma classified as T2. Furthermore, in partial and/or complete clinical responders, other options than the CHEP procedure, such as RT, VPL, and even exclusive chemotherapy, might be used without decreasing survival.

In conclusion, this combined retrospective study from two departments using the CHEP procedure in Europe suggested that the change from the conservative treatment modalities of RT and VPL to CHEP allowed for an increase in long-term survival, local control, and laryngeal preservation. Analysis of the oncologic events after CHEP in patients with a minimum 5-year follow-up demonstrated that the main problem was the incidence and the management of metachronous second primary tumor. We believe that the CHEP procedure should be strongly considered as an option in the initial conservative treatment for patients with glottic tumor presenting fixation or impaired motion of the TVC as long as the arytenoid cartilage is not fixed, the infrahyoid extent of the tumor does not reach the upper border of the cricoid cartilage, and the preepiglottic space is not invaded.

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LOWER CRANIAL NERVE SCHWANNOMAS INVOLVING THE JUGULAR FORAMEN

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Schwannomas involving the jugular foramen are rare lesions, and no consensus exists on their management. This paper reports on 20 such cases treated in our centers. Nineteen cases were operated on for removal of the tumors, and the remaining case is being managed by watchful expectancy. Fifteen cases were operated on by the petro-occipital trans-sigmoid approach with or without labyrinthectomy, 2 by the infratemporal fossa approach, 1 by the modified transcochlear approach, and 1 by the jugulo-petrosectomy approach. The petro-occipital trans-sigmoid approach allowed single-stage, total tumor removal with preservation of the facial nerve and of middle and inner ear functions. Lower cranial nerve paralysis was the major complication and seemed to be inherent to the disease rather than to the approach used. No cerebrospinal fluid leak or meningitis occurred in the present series. So far, no recurrence has been detected.

KEY WORDS — cranial nerve, jugular foramen, schwannoma.

INTRODUCTION

Schwannomas involving the jugular foramen are rare tumors, and only a few centers have relatively large series.[1-6] With advances in radiologic diagnosis and the widespread use of computed tomography (CT) and magnetic resonance imaging (MRI), more and more cases are being diagnosed. Despite their origin from the lower cranial nerves, some cases do not present with preoperative dysfunction of these nerves; they often mimic an acoustic neurinoma or a glomus jugulare tumor.[7,8]

Kaye et al[2] classified jugular foramen schwannomas according to their pattern of growth into three main categories (Table 1[2,5,6,9]): primarily intracranial (type A), mainly involving the bone (type B), and primarily extracranial (type C). Pellet et al[9] added type D, a dumbbell-shaped tumor involving the jugular foramen with both intracranial and extracranial components. This classification was adopted by Samii et al.[6]

Franklin et al[5] preferred a classification similar to that given for glomus jugulare tumors (Table 1). Tumors confined to the neck were classified as class A. Class B tumors were located primarily in the neck, with extension to the jugular foramen. Class C tumors filled the jugular foramen and were further subdivided according to the involvement of the internal carotid artery, and class D was for intracranial extension. The D component was further detailed with subscripts: "e" and "i" to mean extradural and intradural, and "1" and "2" to mean 2 cm or less in diameter, and more than 2 cm. We modified this last classification and added the mention of the neck component. Table 2 shows the modified classification system we advocate.

TABLE 1. CLASSIFICATION OF SCHWANNOMAS OF JUGULAR FORAMEN ACCORDING TO KAYE ET AL,[2] WITH MODIFICATIONS BY PELLET ET AL[9] AND SAMII ET AL[6]; AND ACCORDING TO FRANKLIN ET AL[5]

Class	*(Kaye et al,[2] 1984)*	*Class*	*(Franklin et al,[5] 1989)*
A	Primary intracranial tumor with small extension in bone	A	Tumor confined to neck
B	Primary tumor in jugular foramen with or without intracranial extension	B	Primary tumor in neck with extension to jugular foramen
C	Primary extracranial tumor with extension in jugular foramen or posterior cranial fossa	C	Tumor in jugular foramen; 1 — without expansion to carotid artery; 2 or 3 — with expansion to carotid artery; 4 — with expansion to foramen lacerum
D	Dumbbell-shaped tumor with intracranial and extracranial extension (Pellet et al,[9] Samii et al[6])	D	Intracranial tumor; e — extradural; i — intradural; 1 — up to 2 cm in diameter, 2 — more than 2 cm in diameter

Main component or primary tumor dictates class in Kaye et al, whereas in Franklin et al, both intracranial and bone components are mentioned, and their size and position are specified.

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TABLE 2. OUR SUGGESTED CLASSIFICATION OF LOWER CRANIAL NERVE SCHWANNOMA

Class	*Description*
A	Tumor present in neck, <2 cm in diameter
A+	Tumor present in neck, >2 cm in diameter
C	Tumor in jugular foramen without involvement of petrous internal carotid artery
C+	Tumor in jugular foramen with involvement of petrous carotid artery
D	Intradural tumor <2 cm in diameter
D+	Intradural tumor >2 cm in diameter

Each tumor case is identified with its A, C, or D component or components.

Because of the rarity of these lesions, no consensus exists on their management, as regards the choice of operative approach, and whether or not a one-stage or a staged procedure should be adopted.

This report presents our experience with 20 cases of schwannoma of the lower cranial nerves with involvement of the jugular foramen.

CASE MATERIAL

Twenty-four cases of lower cranial nerve schwannomas were managed in our centers during the period from 1985 to 1995. Two patients had tumors limited to the parapharyngeal space, and 1 to the cerebellopontine angle, and 1 patient with neurofibromatosis-1 had a malignant schwannoma; these 4 cases were excluded from this study. The remainder were 20 jugular foramen schwannomas in 11 women and 9 men, whose ages ranged from 23 to 72 years (mean 42 years). Nineteen patients were operated on, and a 72-year-old man with normal lower cranial nerve function was managed by watchful expectancy and regular clinical and radiologic follow-up.

Table 3 lists the initial symptom in the different cases. The duration of symptoms ranged from 3 months to 13 years, with a mean of 2.6 years. All patients had undergone thorough clinical and neurologic examination with emphasis on preoperative cranial nerve function. Table 4 shows the clinical picture at presentation. All cases were examined by high-resolution CT scan (Fig 1). We performed MRI in 12 cases and angiography in 13.

Three cases presented with recurrent or residual tumor operated on elsewhere by the suboccipital approach. The preoperative diagnosis was correct in 13 primary cases. Two of the remaining 3 were diagnosed as acoustic neurinoma, and 1 was a highly vascular tumor, diagnosed as chemodectoma. This patient had undergone preoperative embolization. Four patients had extradural tumors, and the remainder had extradural and intradural extension.

TABLE 3. INITIAL SYMPTOMS IN LOWER CRANIAL NERVE SCHWANNOMAS (N = 20)

Complaint	*No.*	*%*
Hearing loss	8	40
Dizziness	5	25
Hoarseness of voice	3	15
Swallowing difficulty	2	10
Hemiatrophy of tongue	1	5
Dysphasia	1	5
Shoulder pain	1	5

One of our early cases was managed by a two-stage procedure in which an infratemporal fossa approach type A[10] was used for removal of the extradural part of the tumor, followed by a translabyrinthine approach 3 months later for extirpation of the intradural component. All subsequent patients were managed by a one-stage procedure. In 11, this consisted of a petro-occipital trans-sigmoid approach[11,12] (Fig 2); in 4 other patients presenting severe or total hearing loss, this was enlarged with a posterior labyrinthectomy (Fig 3). An infratemporal type A approach[10] was used in 1 case, a modified transcochlear[13] approach in 1, and jugulo-petrosectomy[14] in the remainder. No postoperative lumbar drains were placed. All patients were followed up with yearly clinical evaluation and CT or MRI to detect recurrence.

RESULTS

Table 5[2,6] lists the tumor type, site, preoperative cranial nerve function, operative management, and postoperative deficits. The normal function of the lower cranial nerves could be preserved in only 3 cases. The remaining patients either did not recover the function of the preoperatively paralyzed lower

TABLE 4. CLINICAL MANIFESTATIONS OF CASES OF LOWER CRANIAL NERVE SCHWANNOMAS (N = 20)

Manifestation	*No.*	*%*
Hearing disturbance	14	70
Total deafness	8	40
Severe SNHL	2	10
Mild to moderate SNHL	4	20
Dizziness or vertigo	9	45
Tinnitus	7	35
Cranial nerve		
VII (paresis)	4	20
IX	9	45
X	8	40
XI	6	30
XII	7	35
Headache	2	10
Retrotympanic mass	1	5
Hemifacial spasm	1	5

SNHL — sensorineural hearing loss.

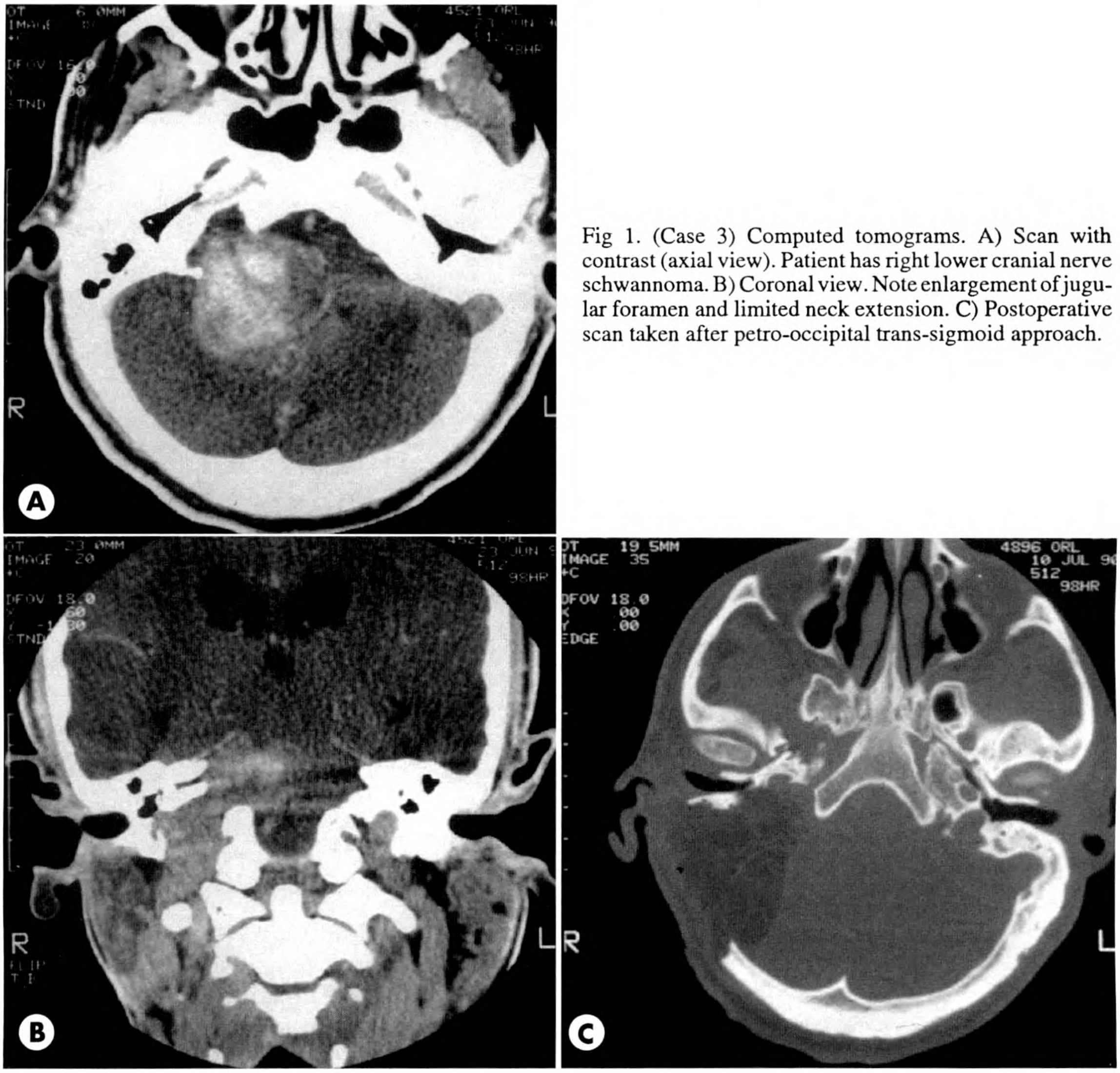

Fig 1. (Case 3) Computed tomograms. A) Scan with contrast (axial view). Patient has right lower cranial nerve schwannoma. B) Coronal view. Note enlargement of jugular foramen and limited neck extension. C) Postoperative scan taken after petro-occipital trans-sigmoid approach.

cranial nerves or developed new paralysis. Eight patients developed a new paralysis of one or more of the lower cranial nerves. This involved the 9th and 10th nerves in 3 cases. The 10th nerve alone was lost in 2 cases, and its loss was associated with the 11th nerve in another patient who had preoperative 9th nerve paralysis. Loss of the 11th nerve occurred in 1 patient who had a preoperative paralysis of the 9th and 10th cranial nerves. Loss of the 9th nerve alone occurred in 1 case. Nasogastric tube feeding was needed in the immediate postoperative period for those who developed paralysis of the 9th and 10th nerves. One patient needed a temporary postoperative tracheostomy because of respiratory difficulty. All the other patients compensated well for their lower cranial nerve loss postoperatively. In no case was paracordal injection of Teflon paste performed.

There was grade 2 facial paresis of case 2, but all the other cases managed by the petro-occipital trans-sigmoid approach coexisted with normal facial nerve function. One patient with normal preoperative hearing, managed by the infratemporal fossa approach type A, suffered total hearing loss. All the other patients with normal hearing or mild hearing loss maintained their preoperative hearing.

There were no cases of cerebrospinal fluid (CSF) leak or meningitis in the present series. Gross total tumor removal was accomplished in all cases. Clinical and radiologic follow-up with CT and/or MRI for 6 months or more is available in 19 cases, with a range from 6 months to 9 years and a mean of 2.3 years. So far, no recurrence has been detected.

SURGICAL TECHNIQUE

Only the petro-occipital trans-sigmoid approach[12] will be described here, since the other approaches have already been reported.[10,13,14]

Under general anesthesia, a lumbar catheter is placed to allow CSF outflow as the dura is exposed.

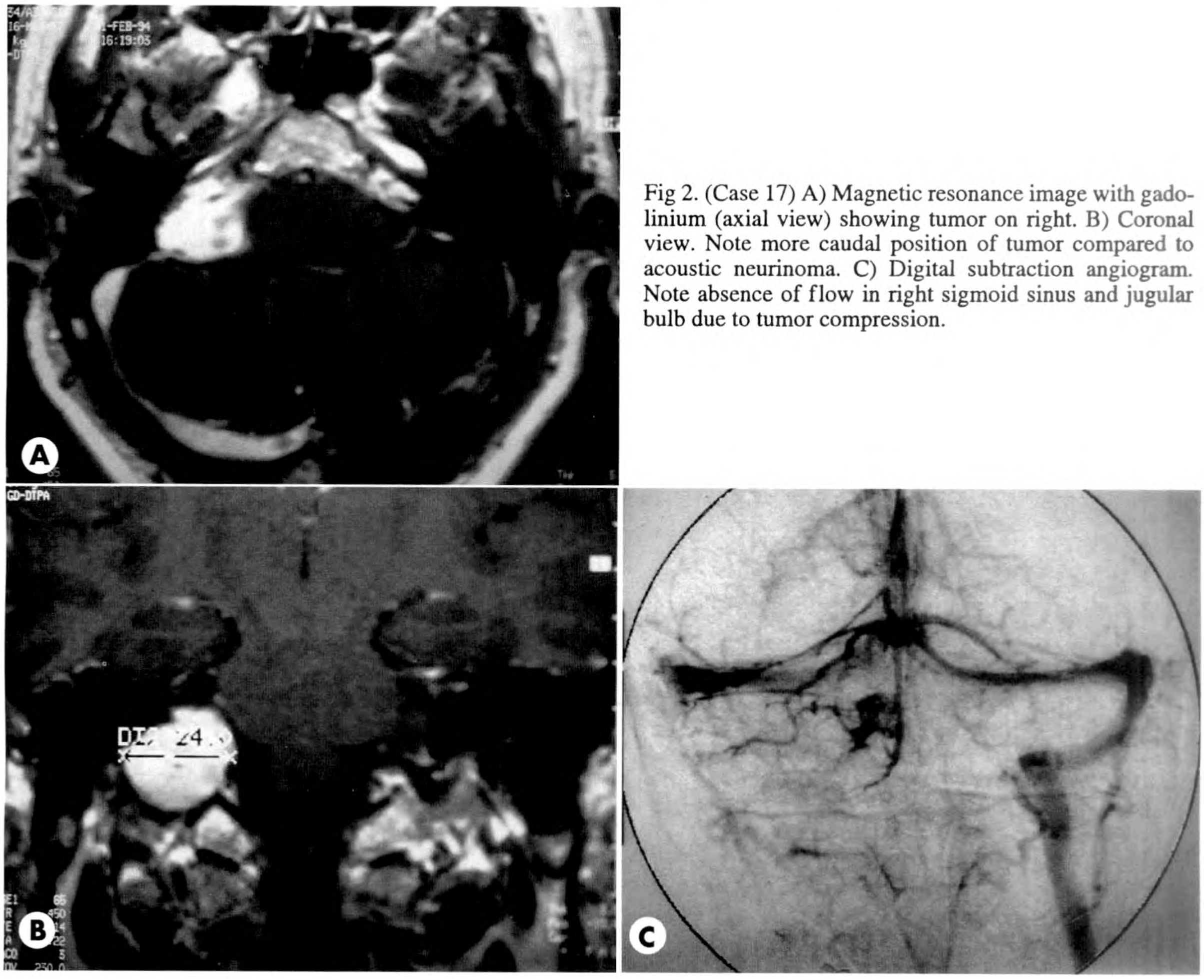

Fig 2. (Case 17) A) Magnetic resonance image with gadolinium (axial view) showing tumor on right. B) Coronal view. Note more caudal position of tumor compared to acoustic neurinoma. C) Digital subtraction angiogram. Note absence of flow in right sigmoid sinus and jugular bulb due to tumor compression.

The patient is put in the supine position with the head turned to the opposite side. A C-shaped incision starting 2 cm above the auricle courses 4 to 5 cm behind the postauricular crease and extends inferiorly to the level of the C-1 vertebra. Its anterior end reaches just posterior to the angle of the mandible. An inferiorly based, myoaponeurotic flap[15] is raised. The base of the flap is at the level of the C-1 vertebra (Fig 4). The sternomastoid muscle is retracted posteriorly and the internal jugular vein is identified just in front of the transverse process of the atlas. The vein is double-ligated, with care not to injure the accessory nerve. An extended mastoidectomy is performed with skeletonization of the sigmoid sinus down to the jugular bulb. The dura is dissected free from the petrous bone and the occipital condyle and depressed medially to allow a better control of the anterior aspect of the field. A 4 × 4-cm retrosigmoid craniotomy is performed. The sinus, the presinus dura, and the jugular bulb are completely uncovered up to the posterior semicircular canal, which is blue-lined and preserved. The infrafacial cells are drilled after identification of the mastoid segment of the facial nerve. Drilling can continue until the vertical segment of the internal carotid artery is identified. The occipital condyle is partially drilled if necessary to the level of the hypoglossal canal. The sigmoid sinus is opened and intraluminally packed with Surgicel (Johnson & Johnson, New Brunswick, NJ). The packing is pushed upstream to the sinus knee and downstream to the jugular bulb. The lateral wall of the sinus is removed. In case of intradural lesions, a transverse incision is made, starting in the retrosigmoid dura, traversing the sigmoid sinus, and continuing in the presinus dura. The incision courses below the level of the endolymphatic sac and reaches down to the jugular foramen (Fig 5). An upper and a lower dural flap are retracted with stay sutures. The jugular bulb is opened. The bulb is usually compressed by the tumor mass. Bleeding from the inferior petrosal sinus is controlled by gentle packing with Surgicel. Tumor removal then starts (Fig 6).

If further control of the internal carotid artery is needed, an extended posterior tympanotomy is performed and the facial nerve from the parotid to the second genu is temporarily mobilized laterally on the posterior wall of the external auditory canal. This

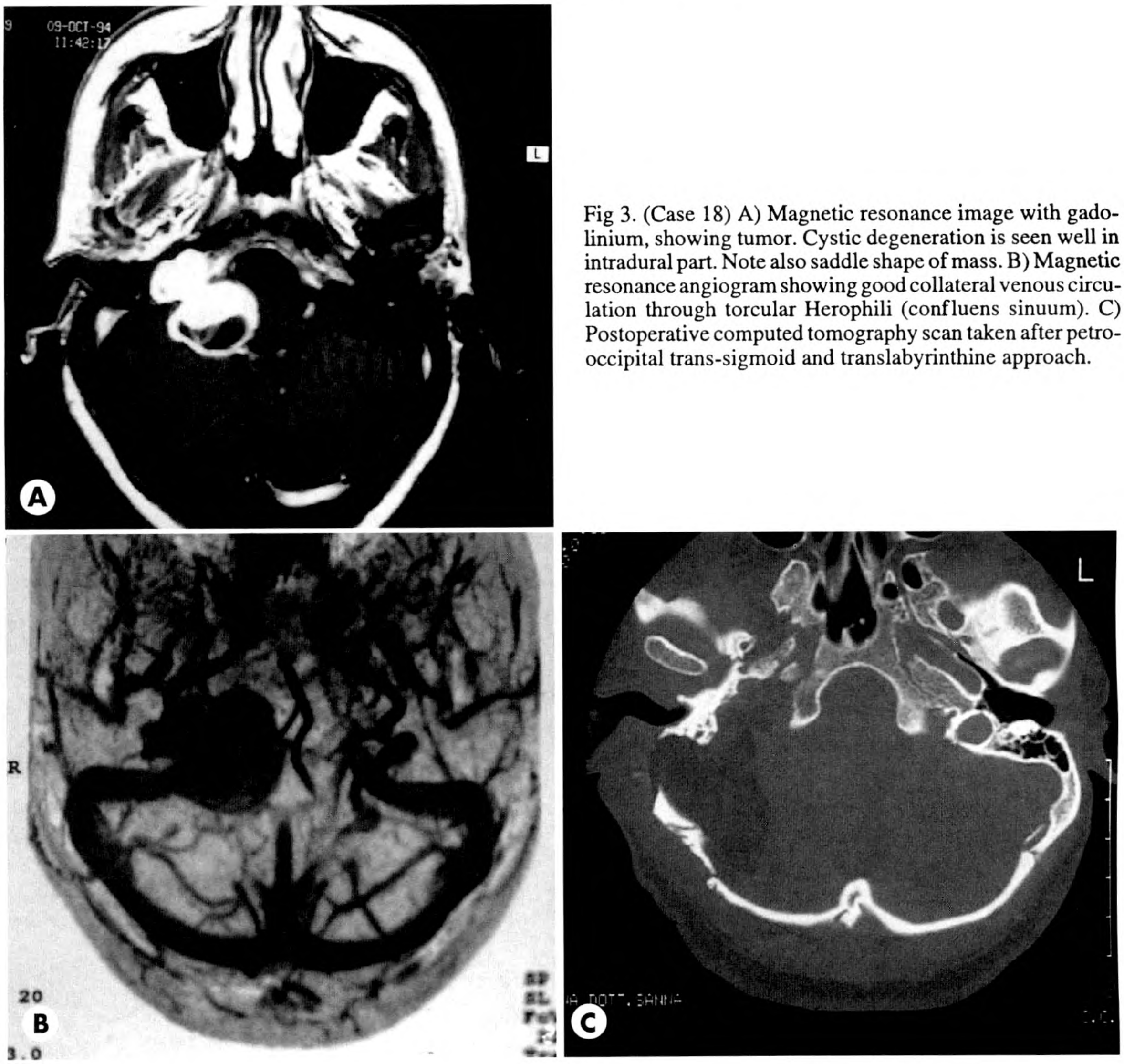

Fig 3. (Case 18) A) Magnetic resonance image with gadolinium, showing tumor. Cystic degeneration is seen well in intradural part. Note also saddle shape of mass. B) Magnetic resonance angiogram showing good collateral venous circulation through torcular Herophili (confluens sinuum). C) Postoperative computed tomography scan taken after petro-occipital trans-sigmoid and translabyrinthine approach.

gives adequate exposure to drill the bone of the posterolateral surface of the vertical portion of the internal carotid artery. Should more control be sought toward the low clivus, the jugular tubercle can be further drilled after retraction of the lower cranial nerves or their sacrifice (if already paralyzed preoperatively). The neck extension of the tumor is dissected and pulled superiorly. This maneuver does not require excessive dissection of the soft tissues of the neck.

At the end of the procedure, the retrosigmoid dura and the medial wall of the sigmoid sinus are approximated with watertight sutures, and the rest of the cavity is obliterated with abdominal fat held in place by the repositioned myoaponeurotic flap. No postoperative lumbar drain is used. No fixation of the craniocervical junction is needed.

DISCUSSION

In view of the location of the jugular foramen and the important structures passing through it, surgical management of tumors of this area poses certain difficulties.[16] These include the frequent association of intracranial and extracranial extension of the tumor, the intimate relation of cranial nerves IX through XI to each other and to the jugular bulb, with the high risk of their injury during surgery, and the close relation of the mastoid segment of the facial nerve to the jugular bulb, being actually centered on it. The fact that many such patients have normal hearing tempts the surgeon to choose an approach that preserves hearing without hampering adequate tumor visualization and control.

Schwannomas involving the jugular foramen can present with diverse clinical features, which can be grouped into three main categories. The first group, encountered in the present series, is a jugular foramen syndrome with paralysis of the lower cranial nerves. The second common clinical picture is a cerebellopontine angle lesion with ipsilateral hearing loss,

TABLE 5. CHARACTERISTICS OF PATIENTS OPERATED ON FOR LOWER CRANIAL NERVE SCHWANNOMA (N = 19)

No.	*Age*	*Sex*	*Hearing (dB)*	*Preoperative Cranial Nerve Deficits*	*Tumor Location*	*Tumor Type**	*Approach*	*Postoperative Nerve Loss*
1	40	F	DE	IX	JF, CC, UN, CPA	D AC+D+	1) IFA, 2) TL	FN grade 3, X-XI
2†	51	F	20	FN grade 2, IX-X	JF, CC	B C+	IFA	XI
3	25	M	DE	FN grade 2	JF, CC, PA, ME, CPA, UN	B AC+D+	POTS	X
4†	39	F	40	IX-X	JF, CPA, UN	D ACD+	POTS	
5	23	M	10	IX-XII	JF, OC, CC, UN, CPA	B AC+D	POTS	
6	44	M	DE	V	JF, CPA	A CD+	POTS + TL	
7	59	F	20	IX-XII	UN, JF	C A+C	POTS + CX	
8	49	M	20	XII	JF, CC, UN	B AC+	POTS	
9	48	F	90	IX, X, XII	JF, CC, PA, CPA	B AC+D+	POTS + TL	
10	40	F	30		JF, CPA	A CD+	POTS	IX, X
11	36	M	DE		JF, CPA	A CD	POTS	IX, X
12	40	F	15	IX-XI	JF, CPA	D ACD	POTS	FN grade 2
13†	46	F	DE	FN grade 2, IX-XII	JF, CPA, UN	D ACD+	POTS	
14	49	F	70		JF, PA, ME, CC, UN	C A+C+	RJP, FN partially mobilized	
15	28	M	DE	IX-XII	JF, CC, PA, CPA, ME, UN	D A+C+D+	TC	FN grade 3
16	54	F	70		JF, CC, CPA§	B AC+D	POTS + TL	FN grade 2, X
17	56	M	20		JF, CC, CPA, UN	B AC+D+	POTS	IX-X
18	34	F	DE	FN grade 2	JF, CPA	A CD+	POTS + TL	
19	60	M	30		JF, CPA	A CD+	POTS	IX

DE — deaf ear, JF — jugular foramen, CC — carotid canal, UN — upper neck, CPA — cerebellopontine angle, IFA — infratemporal fossa type A, TL — translabyrinthine, FN — facial nerve, PA — petrous apex, ME — middle ear, POTS — petro-occipital trans-sigmoid, OC — occipital condyle, CX — cervical, RJP — radical jugulo-petrosectomy, TC — transcochlear.

*First line follows classification systems of Kaye et al[2] and Samii et al,[6] and second line follows our suggested classification (see Table 2).

†Recurrence after suboccipital approach.

§Associated 2-cm acoustic neuroma.

tinnitus, and vertigo, thus simulating acoustic neurinoma. Less frequently, these tumors resemble glomus jugulare tumors with pulsatile tinnitus and a hypotympanic mass.

While patients in earlier series[1,17] frequently presented with manifestations of hydrocephalus and/or cerebellar involvement, we have met no such cases. This reflects physicians' increasing awareness and the widespread availability of radiologic investigations giving earlier diagnosis.

The most important differential diagnosis of these tumors includes chemodectoma, acoustic neurinoma, and meningioma of the jugular fossa.[16-18] High-resolution CT scanning shows smooth widening of

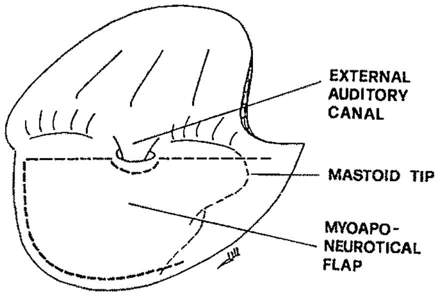

Fig 4. Schematic drawing showing skin flap and myoaponeurotic flap, represented by broken line.

the jugular foramen, with discrete margins and no signs of bony infiltration. In contrast, glomus tumors and meningioma cause irregular bony erosion and infiltration. Angiography shows characteristic rapid filling and intense tumor blush in glomus cases. Such a finding is generally absent in schwannomas.

Tumors situated mainly in the cerebellopontine angle can be differentiated from acoustic neurinoma by their more caudal location. Except for 1 reported case,[19] they do not cause widening of the internal auditory canal.

Gadolinium-enhanced MRI gives a precise definition of the tumor extent (whether extracranial or intradural), and the relationship of the tumor with the internal carotid artery can be studied with accuracy.

Classification of lower cranial nerve schwannomas is the subject of different proposals (Table 1). Kaye et al[2] and Samii et al[6] use the concept of three anatomic compartments — neck, jugular foramen, and posterior cranial fossa — into which the tumor may extend, and classify a tumor by the main compartment it occupies. The Franklin et al[5] classification is more detailed, as it indicates the intracranial and jugular foramen components together with their size, and the intradural or extradural position of the intracranial tumor.

As size, location, and number of components may influence the approach and complications, we prefer the more informative classification of Franklin et al[5] with some modifications (Table 2). Since some of these tumors extend from the cerebellopontine angle to the neck, we add the neck component, as it may influence the surgical strategy and outcome. Second, these tumors have less significant involvement of the petrous bone and the carotid artery than the glomus tumor; therefore, a detailed description of the involvement of the artery as proposed by Franklin et al adds little to the approach chosen or the results of surgery. We feel that a detailed classification covering all tumor patterns should help us to define the management of the different tumors and compare the different views on this subject.

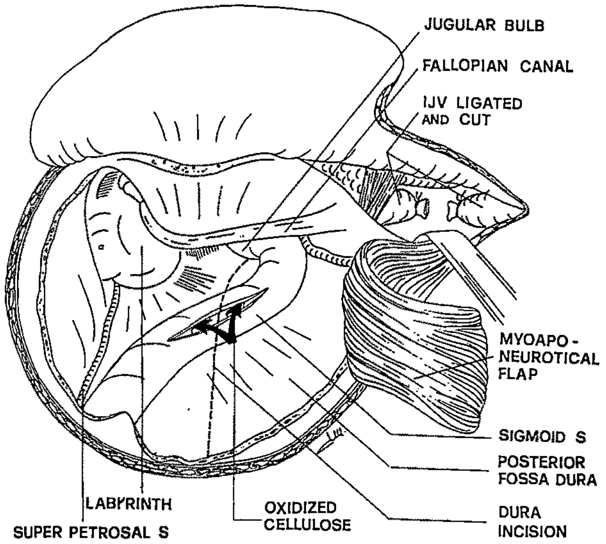

Fig 5. Extradural part of approach is completed. Broken line indicates dural incision. IJV — internal jugular vein, S — sinus.

Total removal is the treatment of choice for lower cranial nerve schwannoma. Table 6[1-7,17,20-26] lists the approaches and outcomes in the main recently published series, including the present one. No general consensus has been reached on the choice of surgical approach, or whether or not the operation should be staged. While some authors[1,4,8,20] used the suboccipital approach, others combined it with an infratemporal fossa approach with facial nerve displacement.[22-24] Kaye et al[2] proposed a combined otological and neurosurgical approach. Horn et al[3] used a variety of procedures, including translabyrinthine or retrolabyrinthine approaches for tumors located mainly intracranially, and an infratemporal approach for cases

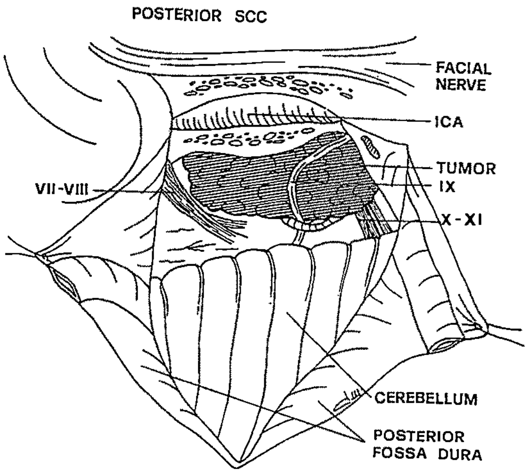

Fig 6. Posterior cranial fossa after incision of dura. Acoustic-facial bundle is dislocated by cranial pole of tumor. Nerves IX and X-XI lie, respectively, on lateral and caudal aspects of tumor. ICA — internal carotid artery, SCC — semicircular canal.

TABLE 6. MAIN RECENTLY PUBLISHED SERIES OF LOWER CRANIAL NERVE SCHWANNOMAS

				Lower Cranial Nerve Deficits (preop/postop)						
Authors	*Year*	*No. of Cases*	*Approaches*	*IX*	*X*	*XI*	*XII*	*FN Paresis*	*CSF Leak*	*Comments*
Arenberg and McCreary[20]	1971	3	SO	1/3	1/3	1/3	0/0	1		Tracheostomy needed
Pluchino et al[1]	1975	12	SO	5/?	3/?	2/?	2/?	1		2 patients died, bronchopneumonia in 3 cases, tracheostomy in 2 cases, gastric hemorrhage in 1 case
Gacek[7]	1976	2	Subtotal petrosectomy	1/1	1/1	1/1	1/1	1		
Call and Pulec[21]	1978	2	Transmastoid + IL + CX + FN partially mobilized	2/2	2/2	2/2	2/2		1	
Hakuba et al[17]	1979	3	1) RLB-trans-sigmoid 2) SO + JF opening	2/3	1/2	1/1	0/0	1		
Crumley and Wilson[22]	1984	4	IFA + SO trans-sigmoid (2 staged)	1/2	1/2	2/2	2/2	2	1	Teflon injection in 1 case
Kaye et al[2]	1984	13	1) PF 2) TL-TC 3) IL (IFA) 4) PF + TL 6 cases staged	?/12	8/13	4/12	2/2	3	1	Teflon injection in 5 cases
Horn et al[3]	1985	7	1) TL 2) RLB 3) IFA 4) RLB-CX	3/4	3/5	2/2	2/2	3		Teflon injection in 3 cases, 6th nerve paresis in 3 cases, recurrence in 1 case
Roland et al[23]	1986	5	1) IF 2) IF + PF 3) IF + PF + TL + TC 4) IF + TL	2/3	2/3	1/2	2/3	2	3	Aspiration pneumonia and Teflon injection in 1 case
Franklin et al[5]	1989	7	IFA 1 case staged	5/5	6/6	5/5	3/3	3°	1	°1 case recovered to grade 2
Sasaki and Takakura[4]	1991	12	1) SO 2) SO + JF opening 3) SO + JF opening + IL 4) IL	9/12	7/10	6/9	3/2	4		Ataxia and hemiparesis in 1 case, 6th nerve paresis in 3 cases, recurrence in 4 cases
Graham et al[24]	1991	2	1) TL 2) SO, IF (staged) 2 cases staged	0/2	0/2	0/1	0/1			Recurrence in 1 case after TL procedure
Mann et al[25]	1991	2	IL + trans-sigmoid	?/?	?/?	?/?	?/?			
Ator et al[26]	1994	2	1) Transmastoid transcervical 2) IF + SO	2/2	2/2	1/2	2/2		1	Teflon injection in 2 cases
Samii et al[6]	1995	16	1) SO 2) Cervicomastoid 3) 1 + 2	8/8	12/12 (+1°°)	6/6	5/5 (+1°°)	3	1	2 cases of mastoiditis; tracheostomy; °°transient deficit
Present series		19	1) POTS 2) TC 3) IF, TL 4) RJP	9/13	8/14	6/8	6/6	4/8°°°		Temporary tracheostomy in 1 case; °°°grade 2 or 3

FN — facial nerve, CSF — cerebrospinal fluid, SO — suboccipital, IL — infralabyrinthine, CX — cervical, RLB — retrolabyrinthine, JF — jugular foramen, IFA — infratemporal fossa type A, PF — posterior fossa, TL — translabyrinthine, TC — transcochlear, IF — infratemporal, POTS — petro-occipital trans-sigmoid, RJP — radical jugulo-petrosectomy.

with neck extension. Samii et al[6] used a suboccipital approach extended to the jugular fossa for tumors located mainly in the cerebellopontine angle, and a cervical transmastoid approach for tumors in the skull base and neck. The sigmoid sinus and jugular bulb were preserved. Franklin et al[5] advocated the infratemporal approach type A for all cases.

In the present series, we used the petro-occipital trans-sigmoid approach in most cases for single-stage tumor removal. In patients with unserviceable preoperative hearing, the approach could include the labyrinth. The basic principle of the petro-occipital trans-sigmoid approach was also utilized by others, but with technical variations.[17,25,27] This approach gave excellent exposure of the jugular fossa, and those portions of the intrapetrous carotid artery that were impacted by an expanding tumor could be controlled well. These were the posterolateral aspect of the vertical segment of the artery in 3 cases and the posteroinferior aspect of the petrous apex segment in 4. In another 3 patients we used the infratemporal type A and transcochlear approaches, as we were not fully aware of the rationale of the petro-occipital trans-sigmoid approach. We had no case of complete encasement of the internal carotid artery, which might have warranted a different approach. Unlike glomus tumors, schwannomas do not tend to infiltrate, so this exposure was usually sufficient for safe tumor dissection. Keeping the facial nerve in situ overcame the problem of residual paresis reported by authors who advocate transposition of the nerve.[4,5,23,24] Sigmoid sinus transection gave optimal visualization of the cerebellopontine angle, while opening the jugular bulb provided adequate control of the jugular foramen. As noted by Mann et al,[25] the jugular bulb is normally already closed by progressive tumor compression, so collateral venous drainage — ascertained by preoperative angiography — is usually well developed. In our cases, the jugular bulb was collapsed by tumor in 12 of 19 cases. Preoperative angiography is mandatory in order to perform a safe closure of the jugular bulb.

Another advantage of the petro-occipital trans-sigmoid approach is the preservation of middle and inner ear function. In the present series, 30% of patients had normal preoperative hearing, and another 20% had only mild to moderate hearing loss. Hearing was preserved in all the patients operated on by the petro-occipital trans-sigmoid approach, who all maintained their preoperative hearing levels. The possibility of preserving hearing in cases of these tumors has been previously noted.[8]

Though the neck exposure by the petro-occipital trans-sigmoid approach is limited compared to the infratemporal fossa or the cervical approach, it is adequate for the relatively safe excision of such noninvasive tumors. The tendency to conservative approaches in lower cranial nerve schwannomas was confirmed by a recent report.[6]

The complications presented in the different series (Table 6) illustrate the problems encountered in surgery of these tumors: mainly, difficulty in preservation of lower cranial nerve function, postoperative CSF leak, and facial nerve functional deficit. Injury of the lower cranial nerves occurred in our series and is probably a problem inherent more to the lesion than to the surgical approach. Preservation of the lower cranial nerves is the current challenge. Intraoperative monitoring of the nerves and a careful technique should yield better results.

A CSF leak was reported in seven of the reviewed series.[2,5,6,21-23,26] Crumley and Wilson[22] attributed this to the single-stage removal of a tumor with intradural and extracranial extension and proposed a two-stage procedure for such cases. Franklin et al,[5] however, advocated a single-stage procedure for all cases whose intradural component was less than 2 cm. After our earlier case 1, we chose a single-stage procedure for all cases irrespective of their intradural size. In agreement with Franklin et al, we found the initial intervention has the best chance for preservation of cranial nerves. Besides the psychologic burden on the patient, scarring from previous surgery would add greatly to the difficulties during a second procedure. It is noteworthy that we had no case of CSF leak despite this strategy. This might be attributed to suturing of the dura and obliteration of the operative defect with abdominal fat, as well as avoidance of excessive dissection and opening of the neck spaces. Keeping the most inferior part of the tympanic bone intact and using the specially designed, inferiorly based myoaponeurotic flap limit the edges of the resulting defect (already reduced by dural suturing) and hold the abdominal fat in place.

We advocate total rather than partial removal.[7] Though total extirpation may entail sacrifice of the nerves involved in most cases, most patients tolerate this paralysis well. Franklin et al[5] noticed this, too. Preoperative paresis of the nerves subsequently sacrificed, the relatively young age of these patients, and the integrity of the contralateral nerves are all important factors in this respect. Incomplete removal, on the other hand, involves inevitable recurrence.[3]

While complete surgical removal is the treatment of choice, it might be prudent to employ a more conservative policy in selected patients. This is illustrated well by the unoperated case in our series.

Sudden palsy of previously functioning lower cranial nerves in old age would be met with serious morbidity. Watchful expectancy is therefore adopted, and surgery is only indicated in case of impending neurologic complications and/or paralysis of the involved nerves.

CONCLUSION

1. Jugular foramen schwannomas are rare tumors that constitute a special entity on account of their location.

2. Clinically, these cases may present with jugular foramen syndrome, or simulate an acoustic neurinoma or a glomus jugulare tumor.

3. We advocate the petro-occipital trans-sigmoid approach in most cases. It has the advantage of excellent exposure, permitting total removal of an intradural and extradural tumor in a single stage with preservation of the middle and inner ear as well as facial nerve function. The cervical and parotid approach is indicated for cervical and extracranial tumors.

4. The most common complications reported in the literature include lower cranial nerve paralysis, CSF leak, hearing impairment, and facial nerve paresis. The last three can be avoided, but lower cranial nerve paralysis remains a problem and seems inherent to the disease rather than to the approach adopted.

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PECULIAR SNORING IN PATIENTS WITH MULTIPLE SYSTEM ATROPHY: ITS SOUND SOURCE, ACOUSTIC CHARACTERISTICS, AND DIAGNOSTIC SIGNIFICANCE

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It is known that abductor paralysis (AP) of the vocal folds sometimes occurs in patients with multiple system atrophy (MSA), and some of them have sleep apnea and loud snoring during sleep. However, the site of obstruction and the sound source of the snoring are still unknown. We performed fiberscopic examinations under diazepam sedation in 8 MSA patients with AP and analyzed the snoring sound. We found that the peculiar snoring occurred with inspiratory vibration of the vocal folds, and there was no obstruction in this portion. Acoustic analysis showed that the fundamental frequency of vocal fold snoring was 260 to 330 Hz, which is different from that of ordinary soft palate snoring. Recognition of vocal fold snoring is important in the early diagnosis of MSA and sleep-related breathing disorders.

KEY WORDS — abductor paralysis, multiple system atrophy, snoring, vocal fold.

INTRODUCTION

Multiple system atrophy (MSA) is a general term that covers Shy-Drager syndrome, olivopontocerebellar atrophy, and striatonigral degeneration. Patients with these diseases have similar symptoms due to cerebellar and autonomic nerve disorders, and pyramidal and extrapyramidal signs. The pathologic findings are also similar. Since it is difficult to distinguish one disease from another, the term MSA was advocated by Graham and Oppenheimer[1] in 1969.

In 1968 Martin et al[2] described patients with idiopathic orthostatic hypotension who had bilateral abductor paralysis (AP) of the vocal folds, and in 1971 Roessmann et al[3] presented an additional pathologic report on the same patients. These two reports seem to be the first to describe MSA with AP. In 1977 Israel and Marino[4] presented a clinical report on Shy-Drager syndrome patients with AP. Since then, there have been several reports on MSA with AP,[5-14] showing that sleep apnea[6-10,14] and peculiar snoring[3-14] occur frequently in MSA patients. Abductor paralysis has been suspected as a cause of sleep apnea and peculiar snoring. However, the fiberscopic appearance of the larynx of these patients during sleep has not been described.

We studied the sound source and acoustic characteristics of peculiar snoring in 8 patients with MSA examined at the Department of Otolaryngology, Osaka Medical College. The diagnostic significance of the peculiar snoring is also discussed.

MATERIALS AND METHODS

Patients. Table 1 shows the profiles of 8 patients with MSA seen in our hospital during the past 10 years. They were 5 men and 3 women, 36 to 65 years old. The chief complaint of all 8 patients was severe snoring. In 5 of the 8 patients (Nos. 2, 3, 4, 6, and 8) MSA had already been diagnosed in the Department of Medicine before they came to the Department of Otolaryngology. In 3 patients otolaryngological examinations suggested MSA. They were referred to

TABLE 1. PROFILES OF EIGHT PATIENTS WITH MULTIPLE SYSTEM ATROPHY

No.	*Age*	*Sex*	*Diagnosis*	*Laryngeal Findings*	*Outcome*
1*	47	M	SDS	Bi AP	Unknown
2	50	M	OPCA	Bi AP	Survival
3	60	M	SDS	Bi AP	Survival
4	36	M	OPCA	Bi AP	Survival
5*	65	F	SDS	Bi AP	Sudden death
6	64	F	SDS	R AP	Sudden death
7*	63	F	SDS	Normal	Survival
8	50	M	OPCA	Normal	Unknown

SDS — Shy-Drager syndrome, OPCA — olivopontocerebellar atrophy, Bi — bilateral, R — right, AP — abductor paralysis.

*Patients receiving diagnosis of SDS after otolaryngological examinations.

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$$\mathrm{PPQ3}=\frac{\dfrac{1}{N-2}\displaystyle\sum_{i=2}^{N-1}\left|P_i-\frac{1}{3}\sum_{n=i-1}^{i+1}P_n\right|}{\dfrac{1}{N}\displaystyle\sum_{i=1}^{N}P_i}\times 10^3$$

$$P=\text{pitch period}$$

Fig 1. Pitch perturbation quotient (PPQ3) of pitch period of voice or snoring sound ("3" indicates number of period peaks needed to calculate numerator). (Modified from Koike.[15])

the Department of Medicine, where the diagnosis of MSA was confirmed.

Laryngeal examination with indirect laryngoscopy revealed vocal fold paralysis in 6 of the 8 patients (Nos. 1-6). Their vocal folds adducted completely to the median position at phonation, but did not abduct fully to the lateral position in the following inspiratory phase. These findings were present during both normal and deep breathing, indicating AP of the vocal folds. Hoarseness was not present in any of the patients.

Acoustic Analysis of Snoring and Respiratory Rate. A cassette recorder (Sony TC-D5M) was used to play back the sound, and acoustic analysis was done with a signal processor (7T17, San-ei).

Ten snores occurring during a stable respiratory period (without apnea or hypopnea) were analyzed for fundamental frequency (F0) and pitch perturbation quotient (PPQ3; formula is shown in Fig 1[15]), and the values for 10 consecutive snores were averaged. The respiratory rate during the same period was also recorded.

Polysomnography. Polysomnography (PSG) was performed the night before fiberscopic examination under diazepam sedation.[16] It included electroencephalography; electrooculography; submental electromyography; measurement of oronasal airflow, chest wall and abdominal movements, and esophageal pressure; and recording of snoring sounds.

Fiberscopic Examination Under Diazepam Sedation. The patients were sedated with diazepam, and pernasal endoscopy was performed for examinations

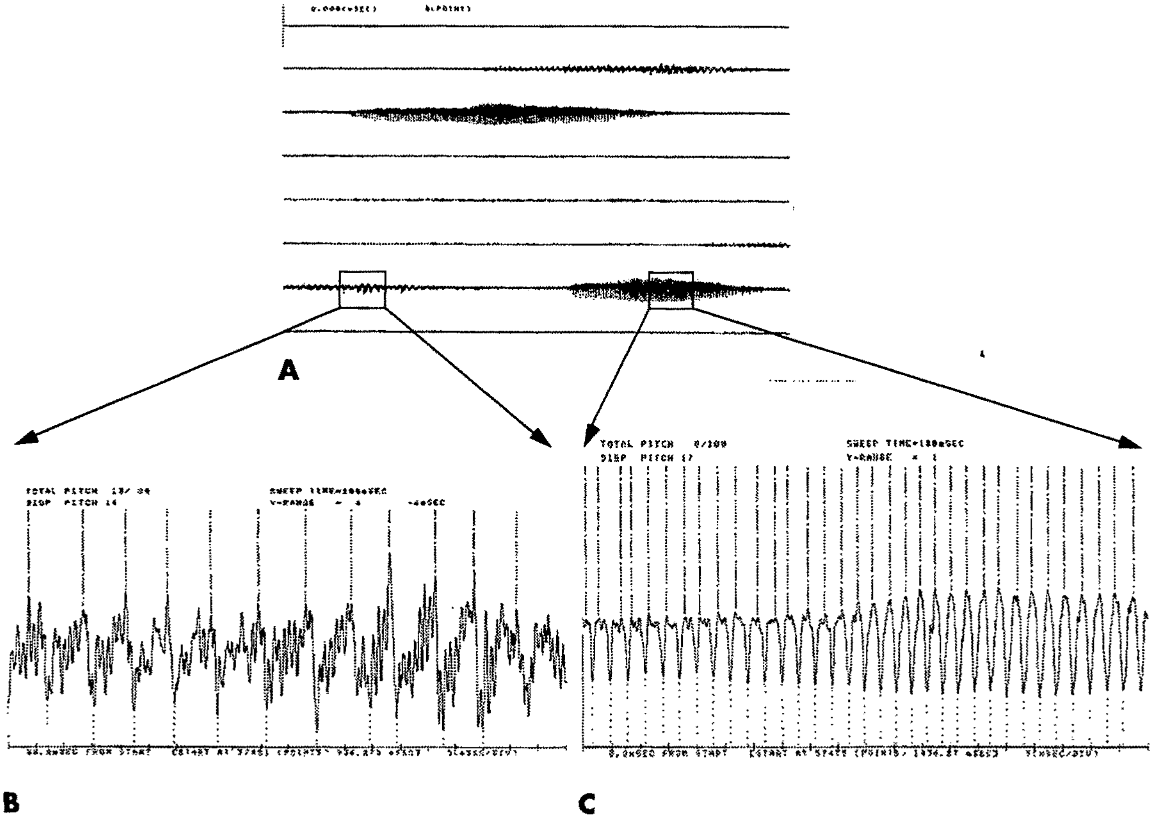

Fig 2. A) Time interval between left side of first line and right side of eighth line is 5 seconds. During this period, about two respirations occurred. Inspiratory phases are from middle of second line to end of third line, and from end of sixth line to end of seventh line. Diphasic snoring is recognized in each inspiratory phase. B) Soft palate snoring occurred in first half of inspiratory phase. Duration is 5 milliseconds. Fundamental frequency (F0) is 136 Hz; pitch perturbation quotient (PPQ3) is 95.0. Interval between broken lines shows pitch period. C) Vocal fold snoring occurred in second half of inspiratory phase. F0 is 326 Hz; PPQ3 is 87.9.

TABLE 2. SUMMARY OF SNORING ANALYSIS

No.	Acoustic Impression	Acoustic Analysis F0 (Hz)	Acoustic Analysis PPQ3	Respiratory Rate (per min)	Apnea Index	Sound Source (Fiberscopic Exam)
1	High pitch, clear	265.4	85.5	29	0	Vocal folds
2	High pitch	271.3	160.9	30	0	Vocal folds
3	High pitch, clear	279.1	26.0	21	0	Vocal folds
4	Low pitch	136.9	95.0	22	3.2	Soft palate
	High pitch, clear	326.7	87.9			
5	High pitch, clear	317.3	36.4	34		
6	High pitch, clear			28	28.7	Vocal folds
7	High pitch, clear	288.8	52.5	28	0	Vocal folds
8	Low pitch	54.3	54.6	15	0	Soft palate

F0 — fundamental frequency, PPQ3 — pitch perturbation quotient, apnea index — apnea numbers divided by hours.

of the upper airway in the supine position during sleep. All findings from this examination were recorded on videotape.

RESULTS

Acoustic Analysis of Snoring and Respiratory Rate. In 7 of the 8 patients (Nos. 1-7), clear, high-pitched sounds were heard in the inspiratory phase. In 1 patient (No. 4), snoring was diphasic (Fig 2A) and consisted of two sounds: a relatively low-pitched sound in the first half of the inspiratory phase (Fig 2B) and a high-pitched sound in the second half of the inspiratory phase (Fig 2C). Snoring in No. 8 was a low-pitched sound in the inspiratory phase. The respiratory rate was increased in all patients except No. 8 (Table 2).

Table 2 shows the results of acoustic analysis. (Snoring of No. 6 could not be analyzed because it was too noisy.) The F0 of the snores of Nos. 1, 2, 3, 5, 6, and 7 and in the second half of the inspiratory phase of No. 4 (Fig 2C) was very high (260 to 330 Hz). The F0 of the snores of No. 8 and in the first half of the inspiratory phase of No. 4 (Fig 2B) was relatively low. The PPQ3s were under 100 in all patients except No. 2.

TABLE 3. SUMMARY OF VOCAL FOLD PARALYSIS AND SAS IN MSA

Authors	Year	Total No.	AP	SAS
Roessmann et al[3]	1971	2	1/2	0/2
Israel and Marino[4]	1977	1	1/1	0/1
Lapresle and Annabi[5]	1978	1	1/1	0/1
Williams et al[6]	1979	12	8/12	2/12
Yabuki et al[7]	1980	1	1/1	1/1
Guindi and Bannister[8]	1981	5	5/5	1/5
Bannister et al[9]	1981	3	3/3	3/3
Hanson et al[10]	1983	12	11/12	2/12
Gilmartin et al[11]	1984	1	1/1	0/1
Kuboshiro et al[12]	1985	1	1/1	0/1
Yasuda et al[13]	1989	10	5/10	0/4
Isozaki et al[14]	1991	48	23/48	6/9
Our cases	1994	8	6/8	1/7
Total		105	67/105 (63.8%)	16/59 (27.1%)

SAS — sleep apnea syndrome, MSA — multiple system atrophy, AP — abductor paralysis.

Polysomnography. We performed PSG in 7 patients, and only No. 6 was seen as having sleep apnea syndrome (SAS).

Fiberscopic Examination Under Diazepam Sedation. This examination was performed in 7 patients. Inspiratory vibration of the vocal fold (vocal fold snoring) was observed in 5 of them (Nos. 1, 2, 3, 6, and 7). Their glottic rimae became very narrow and slitlike during sleep. Inspiratory vibration of the soft palate (soft palate snoring) was observed in No. 8. The glottic rima of this patient was of normal width. In No. 4, vocal fold snoring was heard on a cassette tape, but vibration of the vocal folds was not seen during fiberscopic examination. In No. 7, paralysis of the vocal folds was not observed with indirect laryngoscopy, but the glottic rima became stenotic during sleep, and inspiratory vibration of the vocal folds was observed.

DISCUSSION

It seems that less attention has been paid to sleep apnea, nocturnal tachypnea, and peculiar snoring than to various other symptoms in patients with MSA. However, since sleep disorders are occasionally fatal, as will be mentioned below, more attention must be paid to the nocturnal state of MSA patients.

Sound Source of Peculiar Snoring and Its Acoustic Analysis. Snoring was noted in all our patients, and in 7 of the 8 it differed from ordinary snoring. In 5 of them, stenosis of the glottic rima and inspiratory vibration of the vocal folds were demonstrated with fiberscopic examination under diazepam sedation, so the sound source of this peculiar snoring was the vocal folds. One patient showed no vibration of the vocal folds, and 1 patient died suddenly the night before the scheduled examination, but acoustic analysis suggested that the sound source of the peculiar snoring in these 2 patients was the vocal folds. The speculation of Bannister et al[9] that the sound source of the peculiar snoring of MSA patients is the vocal folds is correct.

Acoustic analysis showed that the F0 of vocal fold snoring was high (260 to 330 Hz), and the PPQ3 was under 100 in almost all the patients.

Motoyama et al[17] found that the F0 of ordinary soft palate snoring was under 170 Hz, and the PPQ3 was much larger than that of vocal fold snoring. On the other hand, the F0 of stenotic snoring, which occurs when the airstream is forced to pass through a stenotic space due to tonsillar hypertrophy or glossoptosis, ranges from 300 to 500 Hz, and the PPQ3 is much larger than that of soft palate snoring.

The average F0 of ordinary vocal fold vibration during conversation in normal Japanese is about 120 Hz in men and 240 Hz in women. The PPQ3 is under 10.

Therefore, the acoustic characteristics of vocal fold snoring are different from those of normal snoring or voice sounds. However, the reasons that vibration of the vocal folds occurs only during inspiration and that the F0 of men and women does not differ as it does in vocal fold vibrations during speech are subjects needing further study.

Nocturnal tachypnea was noted in our 7 patients with vocal fold snoring, although their waking respiratory rate was normal. In patient 8, who had no nocturnal tachypnea, vocal fold snoring did not occur, while soft palate snoring was present. Therefore, it is quite possible that the nocturnal tachypnea in patients with MSA is the result of glottic stenosis rather than of any central disorder.

Diagnostic Significance of Vocal Fold Snoring. Vocal fold snoring is not only of acoustic interest, but also of diagnostic use. First, vocal fold snoring suggests AP of the vocal folds. It is different from bilateral or unilateral recurrent nerve paralysis, so AP due to MSA does not cause hoarseness, because the vocal folds adduct enough in phonation, and it does not cause severe dyspnea, because the glottic rima is relatively wide during inspiration. Therefore, vocal fold snoring is the only clue to vocal fold paralysis. In our study, AP was easily detected by indirect laryngoscopy in 6 of 7 patients who were acoustically presumed to have AP while awake.

Table 3[3-14] lists 105 cases of MSA reported, including our 8 patients. Vocal fold paralysis was found in 67 patients (63.8%). This means that AP is a very common finding in MSA patients.

Second, vocal fold snoring alerts us to sleep-related breathing disorders. As mentioned above, patients with MSA without any breathing disorders while awake show nocturnal tachypnea due to glottic stenosis, although the reason that glottic stenosis occurs only during sleep is unknown.

The most serious problem for MSA patients is nocturnal sudden death. Two of our 8 patients, Nos. 5 and 6, for whom a tracheostomy was scheduled, died suddenly during sleep. The cause of death was probably respiratory arrest. Previous papers have reported sudden deaths of many MSA patients with or without tracheostomy.

Of our 8 patients, only 1 (No. 6) was found to have SAS by PSG, and nasopharyngeal obstruction was observed fiberscopically under diazepam sedation. In previous reports, SAS was recognized in 16 of 59 patients (27.1%; Table 3). The incidence of SAS in the general population is 1% to 3%,[18] so an incidence of 27.1% seems very high. The glottis was the suspected site of obstruction.[10] However, that seems unlikely, in light of our experience.

Third, vocal fold snoring may be the earliest symptom of MSA. Actually, vocal fold snoring led to the diagnosis of MSA in 3 of our 8 patients. Although a relationship between MSA and AP (or vocal fold snoring) is not definite even now, it is very probable, because the incidence of vocal fold paralysis in MSA patients is 63.8%.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

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EFFECT OF ADULT-ONSET DEAFNESS ON THE HUMAN CENTRAL AUDITORY SYSTEM

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Degenerative change in the central auditory system was assessed in seven subjects with profound bilateral adult-onset deafness. The degree of transneuronal atrophy was determined by measuring cell size at three levels of the brain stem auditory pathway (anteroventral cochlear nucleus, medial superior olivary nucleus, and inferior colliculus). Within subjects, the relative degree of cell shrinkage was similar across all levels of the central pathway. Across subjects, the best neuronal preservation was seen in a case of viral labyrinthitis with 1 year of bilateral deafness and a near-normal population of cochlear ganglion cells. Reduction in cell size was greatest in cases of bacterial labyrinthitis or Scheibe degeneration with reduced populations of ganglion cells and longer periods of deafness. At the level of the cochlear nucleus, there was no consistent difference in cell size between the side stimulated by a functioning prosthetic device and the nonstimulated side.

KEY WORDS — cochlear nucleus, hearing loss, inferior colliculus, superior olive, transneuronal atrophy.

INTRODUCTION

Severe degeneration of the central auditory pathway is likely to limit the benefit that can be obtained from any type of auditory prosthesis, but little is known about the changes occurring in the human nervous system after the onset of profound deafness. Information is particularly limited in regard to central changes occurring after deafness with onset in adolescence or adulthood,[1] as opposed to syndromes involving congenital deafness.[2,3] The goal of the present study was to determine the severity of transneuronal atrophy in central auditory nuclei in a population of subjects with profound bilateral adult-onset deafness. Because the subjects were part of a clinic-based temporal bone and brain stem donor program, it was possible to correlate the data on neuronal preservation with clinical histories, audiologic and surgical records, and postmortem histopathologic evaluations of the cochlea.

In this study, we examined the brain stems of seven subjects with bilateral profound deafness beginning during their teen or adult years. Five of these subjects had been included in a previous study of neuronal size in the posterior ventral cochlear nucleus.[4] In the present investigation, we looked at neuronal size in the anterior ventral cochlear nucleus (AVCN), medial superior olive nucleus (MSO), and central nucleus of the inferior colliculus (CIC). The cochlear nuclei are the point of termination of all cochlear nerve axons. Cells in the cochlear nuclei project bilaterally to the superior olivary nuclei for correlation of binaural input. Both cochlear nucleus and superior olivary neurons project to the inferior colliculus. Thus, study of these three nuclei examines neurons that are separated by one, two, and three synapses, respectively, from the eighth nerve. As a measure of transneuronal atrophy, we determined cross-sectional area of neurons in the AVCN, MSO, and CIC. Previous experimental studies have shown that auditory neurons become smaller when deprived of auditory input.[5-9] Further, this reduction in cell size has been shown to be correlated with a reduction in metabolic rate and lowered protein production.[10-12] Thus, cell size is an appropriate index of central degenerative change in both a structural and a functional sense.

MATERIALS AND METHODS

Subjects. Measurements of cell size were made in the brain stems of seven subjects with known clinical histories of deafness (see Table). Each of the seven subjects became profoundly deaf bilaterally during adolescence or adulthood. Three of the subjects had

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SUBJECTS AND CONDITIONS OF DEAFNESS

Subject	*Cause*	*No. of Ganglion Cells*	*Period of Deafness (y)*	*Age at Death (y)*
88-08	Idiopathic sensorineural	R 10,000 L 14,000	R 5, L 5	62
90-46	Scheibe degeneration	R 0 L 8,370	R 45, L 23	70
90-49	Scheibe degeneration	R 8,838 L 9,882	R 26, L 26	61
91-17	Idiopathic labyrinthitis	R 19,764 L 32,364	R 1, L 1	42
91-24	Bacterial meningitis	R 4,194 L 3,180	R 3, L 3	77
92-11	Bacterial meningitis	R 8,463 L 3,212	R 31, L 31	54
92-13	Postoperative neuromas	R 0* L 0*	R 10, L 6	37

*Cochleas normal but surgically disconnected from brain stem.

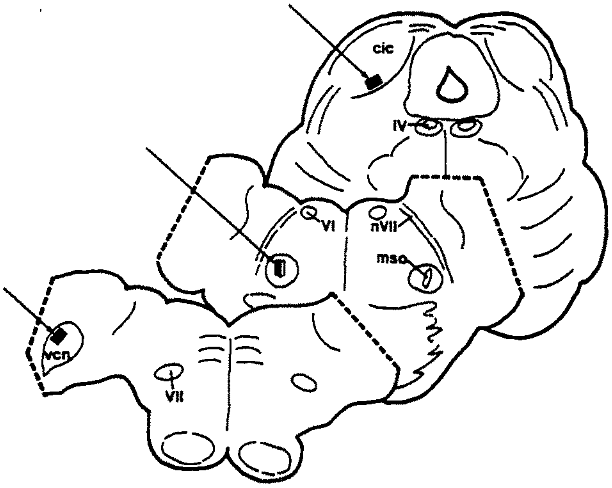

Fig 1. Axial sections through brain stem at level of cochlear nuclei, superior olivary complex, and inferior colliculus. Solid rectangles indicate areas of sampling in anterior ventral cochlear nucleus (vcn), medial superior olivary nucleus (mso), and central nucleus of inferior colliculus (cic). Cranial nerve structures are labeled for reference: IV — trochlear nucleus; VI — abducens nucleus, VII and nVII — facial nucleus and facial nerve.

inflammatory conditions (two cases of bacterial labyrinthitis secondary to meningitis, one case of probable viral labyrinthitis). One subject had idiopathic progressive sensorineural hearing loss, one lost hearing following removal of bilateral acoustic neuromas caused by neurofibromatosis 2 (NF2), and the remaining two cases were diagnosed as adult-onset Scheibe degeneration on the basis of postmortem temporal bone histopathology. In six cases, the period of total deafness for each ear was viewed as beginning at the time of a no-response audiogram (loss >95 dB) and is thus a conservative estimate of the period of hearing loss. In the NF2 subject, the onset of total deafness occurred at the time of surgical removal of the tumors. Periods of bilateral deafness ranged from 1 year to 31 years. Five of the subjects had received cochlear implants, and the NF2 subject received a brain stem auditory implant. The brain stem electrode and four of the cochlear implants were functional, and all of these recipients were termed average users.

Histology and Morphometry. In all cases, the temporal bones and brain stem were obtained and fixed between 6 and 24 hours after death. Six of the brain stems were obtained through the House Ear Institute temporal bone donor program, and one (88-08) was obtained through the Veterans Administration Hospital, Ann Arbor, Mich. Temporal bones were histologically processed and given a postmortem histopathologic diagnosis. As part of this diagnosis, the number of ganglion cells was counted in every 10th slide and the total number of cells was calculated by the Konigsmark formula.[13] In one case (88-08), thinness of sectioning precluded ganglion cell counts, and estimates were made of the remaining cell populations by two independent observers. In the subject with bilateral acoustic neuromas (92-13), surgical removal of the tumors had disrupted all input from the inner ear to the brain stem. In this case, the effective number of ganglion cells was considered to be zero.

Brain stems were embedded in celloidin and sectioned at 75 µm. Adjacent series of every 10th section were stained by the Nissl method for cells and the Woelcke method for myelinated fibers. After staining, all brain stems were assessed for normality of size and staining quality of nonauditory neurons. To determine the size of auditory neurons, measurements were made in the AVCN, MSO, and CIC on both sides of the brain for each subject. The only exception was subject 92-11, in whom the brain stem tissue block did not include the inferior colliculus. Consistent locations for sampling were selected within the three nuclei (Fig 1). At each site, neurons were sampled within a 1.24-mm^2 rectangular area. Within this area, the profile of every cell with a visible nucleolus was traced at 600×, by means of a Nikon Optiphot microscope equipped with a drawing tube. Each tissue block contained between 40 and 70 neurons with nucleoli, except in the cases of Scheibe degeneration. In these two subjects, cells were sparse and only 25 to 40 cells could be traced, but since enlarging the sample area often involved moving into a different cytoarchitectonic region of the nucleus, the size of the sample area was kept constant. Once cell profiles were traced, they were digitized into the PC3D system (Jandel), which determined mean and SD for each sample. Normal mean size for AVCN, MSO, and CIC neurons was determined by similar measurements made on the brain stem nuclei of six

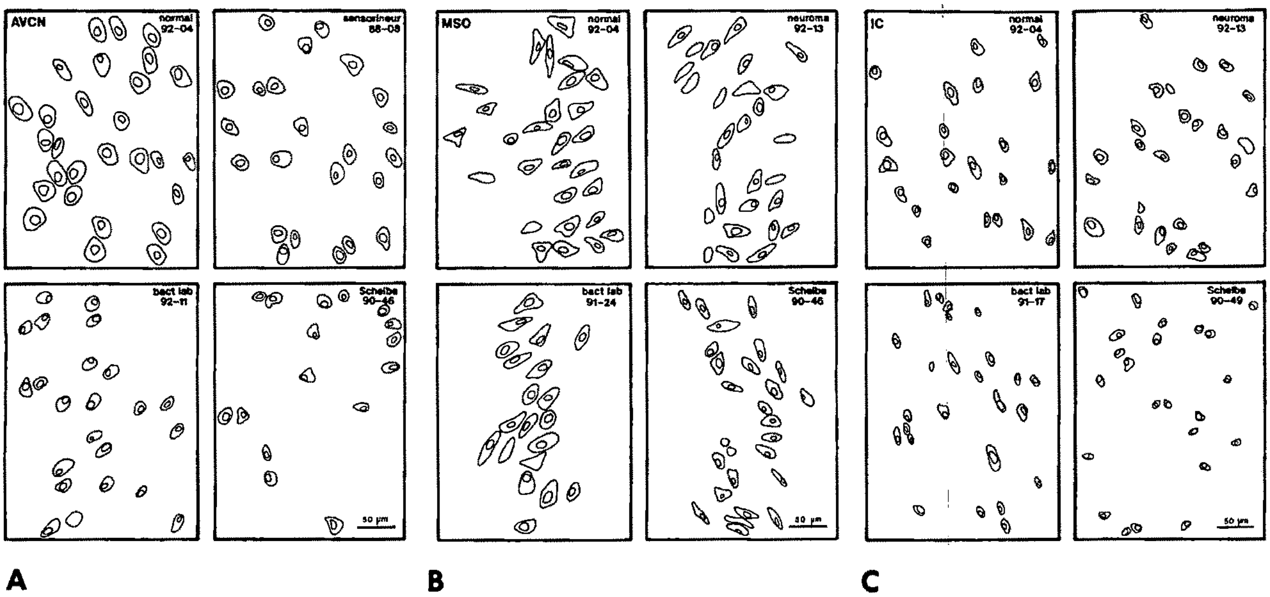

Fig 2. Profiles of neurons in hearing subject and three subjects with profound hearing loss. A) In anterior ventral cochlear nucleus (AVCN). B) In medial superior olivary nucleus (MSO). C) In central nucleus of inferior colliculus (IC).

subjects, ranging in age from 35 to 78 years, who had no history of hearing loss. Data from these six subjects were pooled to produce a normal mean for each of the three nuclei.

RESULTS

Representative drawings of neuronal profiles and graphs of cell size are shown for each of the central nuclei studied, ie, the AVCN (Figs 2A and 3A), the MSO (Figs 2B and 3B), and the CIC (Figs 2C and 3C). In each of the composite drawings (Fig 2), the upper left panel shows a portion of the nucleus in a 46-year-old hearing subject to illustrate normal neuronal size and density. The other panels in each composite are taken from the nuclei in three of the deaf subjects, and illustrate various degrees of cell shrinkage. In these Figures it should be noted that normal neurons in the AVCN and MSO are considerably larger than those in the CIC.

In the cell size graphs (Fig 3), the subjects are arranged in order of average cell size (mean of the right and left sides), but the values for the right and

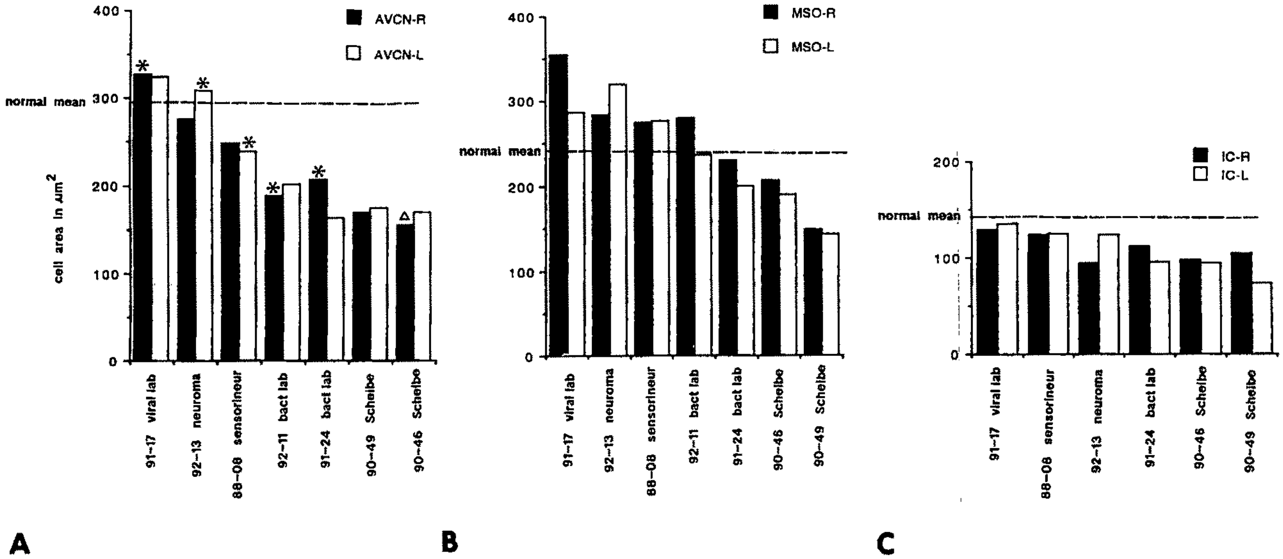

Fig 3. Graph of mean cell size in seven deaf subjects, ordered from largest to smallest average size. Mean cell size in six hearing subjects is indicated by dotted line. Etiology indicated as follows: viral lab — probable viral labyrinthitis; neuroma — acoustic neuromas, NF2; sensorineur — idiopathic sensorineural hearing loss; bact lab — bacterial labyrinthitis, secondary to meningitis; Scheibe — Scheibe degeneration, adult onset. A) In AVCN. Side of auditory prosthesis (cochlear or brain stem implant) is indicated by asterisk, and nonfunctional cochlear implant by triangle. B) In MSO. C) In IC.

left sides are displayed separately. Each graph also displays mean normal cell size derived from the pooled measurements in six normal-hearing subjects. In the case of the AVCN (Fig 3A), the side of a functional auditory prosthesis (cochlear or brain stem implant) is indicated by an asterisk, and the nonfunctional cochlear implant by a triangle. Because neurons on the side of prosthetic stimulation are not consistently larger or smaller than on the unstimulated side, no statistical test was run on cell size in the stimulated and unstimulated cochlear nuclei. Because the MSO and CIC are bilaterally innervated, there is no stimulated or unstimulated side at the level of these nuclei.

In the CIC, neurons in the worst-affected subjects are approximately 30% smaller than those in the least-affected subjects. In the AVCN and MSO, the difference between the largest and smallest neurons reaches 50%. Since neuronal size reduction has been shown to be a loss of cell cytoplasm, with little change in nuclear size,[5] it is possible that cells that initially have more voluminous cytoplasm, such as those in the VCN and MSO, will show a proportionately greater reduction in size. However, within any one subject, the relative degree of change appears to be similar across the three central nuclei. This is reflected in the fact that the position of the subjects in the graphs of cell size (Fig 3) is similar for each of the three nuclei.

Like cell size, staining quality was very uniform across the nuclei within each subject. The Nissl stain is based on a basic aniline dye that binds to nucleic acids in the nucleus and cytoplasm of the cell. Intensity of Nissl staining, when compared to the normal level for a given neuronal population, is an indicator of the amount of nuclear and cytoplasmic RNA present, and is thus a rough indicator of the level of a cell's metabolic activity. The virtually identical change in staining across the three nuclei in each subject implies that the degree of reduction of cell metabolic activity is quite uniform along successively higher auditory nuclei in the brain stem pathway.

In contrast to the uniformity of change within any given subject, we observed differences in degenerative change across subjects. Inspection of the cell size graphs reveals that neuronal size in the deaf subjects ranges from close to the normal mean to considerably below it. At each level of the brain stem, the subjects with viral labyrinthitis, sensorineural hearing loss, and acoustic neuromas have the best preservation of neuronal size. Greater reduction in cell size is seen in the two subjects with bacterial labyrinthitis, and the smallest cells are found in the two subjects with Scheibe degeneration. Like cell size, the intensity of Nissl staining was most reduced in the cases of bacterial labyrinthitis and Scheibe degeneration, indicating low levels of cytoplasmic RNA.

DISCUSSION

The observations in this study make it clear that peripheral dysfunction affects all levels of the brain stem auditory pathway. This implies that the constellation of factors negatively influencing central neuronal status operates across one or more synapses in the auditory pathway, and not simply at the point of termination of eighth nerve axons in the cochlear nuclei. These observations in human subjects differ somewhat from the findings in experimental studies of animals, which tend to show very slight size reduction in central neurons after hearing loss during adulthood.[8,9,14] One factor that may account for this difference is the extremely short survival times, often only 1 to 2 months, used in the animal studies. When a survival period of 1 year was used, the findings in cats were similar to ours in showing 20% to 30% reduction in cell size in the denervated cochlear and olivary nuclei.[5]

We observe that the degree of degenerative change in cell size and morphology is similar across the brain stem nuclei in any single subject, but different across subjects. To what factors might these intersubject differences be attributed? One factor to be considered is differential survival of cochlear ganglion cells. Studies in animals have consistently shown that cochlear destruction, with associated loss of spiral ganglion cells, has more severe effects on central neurons than sound deprivation with an intact cochlea.[5,8,9,11,14] The population in this study demonstrates various degrees of ganglion cell loss, with the total number of residual ganglion cells ranging from a near-normal level to effectively none. At one extreme, the case of viral labyrinthitis has a near-normal ganglion cell count (20,000 and 32,000) and the best maintenance of cell size. The case of sensorineural deafness, with 10,000 and 14,000 ganglion cells, also shows good neuronal survival. In contrast, neuronal atrophy is more pronounced in the cases of bacterial labyrinthitis and Scheibe degeneration, in whom ganglion cell counts were generally between 3,000 and 9,000.

The negative effect of loss of ganglion cells on central neurons is believed to be due to brain stem deafferentation, as cochlear nerve axonal firing and release of transmitter-related substances is assumed to have a trophic effect on AVCN neurons. This assumption is supported by studies involving tetrodotoxin (TTX), a sodium channel blocker. Experiments in which TTX is delivered to the labyrinth demonstrate an electrically silent nerve and an immediate reduction in size, metabolism, and protein incorporation in AVCN neurons.[11,12,15] In our deaf subjects, firing of cochlear nerve fibers could have resulted either from spontaneous activity of the residual hair cells or from cochlear prosthetic stimulation. We do not, however, observe any consistent differences between the implant-stimulated and unstimulated AVCNs. The observed trend toward greater neuronal change in cases with fewer surviving ganglion cells could reflect reduced brain stem innervation, but at present, we are not able to accurately determine the number of surviving axons in the cochlear nerve.

Another factor that could influence central degenerative changes is the time period of profound deafness, particularly since clinical studies have associated long duration of acquired deafness with reduced benefit from cochlear implantation.[16,17] In our population, the subject with the shortest period of bilateral deafness (1 year) has the best maintenance of neuronal size, but as mentioned above, also has the largest population of ganglion cells. Three of the four cases with greatest degeneration had the longest periods of bilateral deafness (23, 26, and 31 years), but these cases also have severely reduced ganglion cell populations. A case of bacterial labyrinthitis with only 3 years of deafness shows badly degenerated auditory nuclei, and this subject also has relatively few surviving ganglion cells (3,000 and 4,000). Given this overlap in conditions, we are not able to separate the effect of the duration of deafness from that of the loss of cochlear ganglion cells.

A notable exception to the correlation between number of ganglion cells and maintenance of central neurons is the NF2 subject, in whom there is no eighth nerve on either side subsequent to bilateral neuroma excisions, but good preservation of neuronal size. If the eighth nerve exerts a trophic effect, the NF2 subject should have the worst central atrophy, but this case shows excellent preservation of auditory neurons. The NF2 case is also unique in the sense that the schwannoma, being a nerve sheath tumor, does not involve a primary disorder of the cochlea or ganglion cells. Our observations would be better explained by the absence of a "dystrophic" influence exerted on the brain by a diseased cochlea than by a lack of a trophic influence from eighth nerve innervation.

CONCLUSIONS

It is clear that central degenerative change can occur in profound deafness, and that the neuronal changes are quite similar across levels of the brain stem in any given subject. Intersubject changes confirm the intuitive expectation that less atrophic change occurs in the central auditory system with shorter survival time and large numbers of ganglion cells, while a long period of deafness and severe loss of ganglion cells has a more negative effect on central auditory neurons. However, even in the worst cases in our population, there are many viable neurons present for up to three decades after onset of bilateral deafness. The case of NF2, with complete interruption of both auditory nerves, is an interesting exception to the notion of a trophic effect of ganglion cells, and raises the possibility that cochlear disease may play some role in determining the degree of negative effect of deafness on the central auditory system.

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REVERSIBLE UREMIC DEAFNESS: IS IT CORRELATED WITH THE DEGREE OF ANEMIA?

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Hearing loss is a common finding in patients with end-stage renal failure. Uremic toxins, ototoxins, and axonal uremic neuropathy appear to be likely pathogenic factors. We analyzed whether an improvement in hearing capacity can be achieved with an improvement of anemia by erythropoietin (EPO) administration. Fifty patients on long-term hemodialysis in a single center were examined audiologically by otoscopy, tympanometry, pure tone audiometry, and the short increment sensitivity index. Twenty-five patients were treated with EPO in a dose of 120 U/kg per week over a period of 5 to 8 months, and the remaining 25 patients were not treated with EPO (controls). Both groups were reexamined audiologically after the study period, and the results were compared. In the group treated with EPO, the hemoglobin level increased from 7 ± 0.9 to 11 ± 0.8 g/dL, as against the control group, whose hemoglobin increased from 7.1 ± 0.9 to 8 ± 0.8 g/dL. The audiologic tests were repeated at the end of the study period, and a significant improvement of hearing was found in the patients treated with EPO as compared with the control group ($p < .001$). Our study suggests that improvement of anemia in patients on long-term hemodialysis by administration of EPO is associated with an improvement in hearing capacity in a significant number of patients. Thus, anemia seems to be an important factor responsible for hearing disorders in patients with end-stage renal failure. Studies with larger numbers of patients are required to confirm this observation.

KEY WORDS — anemia, deafness, end-stage renal disease, erythropoietin.

INTRODUCTION

Hearing loss has been frequently observed in patients with end-stage renal failure (ESRF).[1-9] This disorder seems to be due to a lesion in the hair cells of the cochlea and it is reversible after successful renal transplantation.[10-12] However, it is not clear whether uremic toxins or other factors like anemia are responsible for this reversible lesion of the cochlea. To clarify this problem, a group of patients with ESRF and severe anemia on chronic hemodialysis (HD) were examined by audiometric tests before and 5 to 8 months after erythropoietin (EPO) administration in order to determine whether improvement of anemia would result in improved hearing.

PATIENTS AND METHODS

A total of 50 patients on long-term HD were investigated. They were taken from a group of over 400 patients who were on maintenance HD at the Jeddah Kidney Center. All the patients met the following inclusion criteria: 1) history of hearing impairment, 2) on HD at our center for over 3 years, 3) mean hemoglobin (Hb) of 7 g/dL, and 4) expected to stay on regular schedule of HD for the following 8 months. Patients with a family history of primary otological disease and those with Alport's syndrome, conductive deafness, or hyperparathyroidism were excluded from the study.

The study patients (n = 50) were then randomized into two groups. Twenty-five patients were treated with EPO at a dosage of 120 U/kg per week administered subcutaneously for 5 to 8 months. The remaining 25 patients were not given EPO and served as the control group. Both groups of patients were subjected to detailed audiologic examination. The tests performed included otoscopy, tympanometry, tone audiometry, and the short increment sensitivity index (SISI) test. The SISI is an audiologic test that assesses

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TABLE 1. EFFECT OF EPO TREATMENT ON HEARING CAPACITY IN PATIENTS ON HEMODIALYSIS (N = 25)

	Before EPO	*After EPO*
Hemoglobin level (g/dL)	7 ± 0.9	11 ± 0.8
Hearing capacity		
SND (total)	14/25 (56%)	2/25 (8%)*
Positive SISI score	17/25 (68%)	4/25 (16%)*

Twelve of 25 patients with sensorineural deafness (SND) improved after treatment with erythropoietin (EPO). Improvement in short increment sensitivity index (SISI) score was seen in 13 of treated patients.

*p < .001.

TABLE 2. HEARING CAPACITY IN PATIENTS ON HEMODIALYSIS NOT TREATED WITH EPO (N = 25)

	Start of Study	*End of Study*
Hemoglobin level (g/dL)	7.1 ± 0.9	8 ± 0.8
Hearing capacity		
SND (total)	14/25 (56%)	14/25 (56%)*
Positive SISI score	16/25 (64%)	16/25 (64%)*

EPO — erythropoietin, SND — sensorineural deafness, SISI — short increment sensitivity index.

*p < .001.

the cochlear function in an indirect way through assessing the ability of the ear to detect minute changes in intensity and considering that as indirect evidence for the presence of recruitment. These tests were performed twice: once at the beginning of the study and once after 8 months, at the end of the study period. Since the audiogram results are known to vary during dialysis treatment, this test was performed at the end of 24 hours of dialysis for each patient. The two study groups were matched as to age, sex, SISI score, and duration on dialysis. Patients from both groups received similar dialysis prescriptions, and the prestudy parameters, including blood urea, serum creatinine, and electrolytes, were comparable. Thus, all the baseline parameters, including the extent of hearing loss, were comparable between the two groups. For statistical analysis, *t*-tests for both paired and unpaired samples were used.

RESULTS

A total of 50 patients were studied. Among the 25 patients who were administered EPO, the mean Hb level increased from 7 ± 0.9 g/dL before EPO treatment to 11 ± 0.8 g/dL after 8 months of treatment. Fourteen of the 25 patients (56%) had sensorineural deafness (SND), and 17 of the 25 patients (68%) had high SISI scores at the commencement of the study. At the end of the study period, 12 of the patients with SND and 13 of the patients with high SISI scores showed improvement concomitant with the rise in Hb level (Table 1).

In the control group, the mean Hb level increased from 7.1 ± 0.9 to 8 ± 0.8 g/dL. None of the patients with hearing loss in this group showed improvement upon reexamination after 8 months (Table 2). The improvements of both the SND and SISI scores between the treatment and control groups were statistically significant ($p < .001$). The only other hematologic parameter that was altered by EPO therapy was the increase in total white cell count. The dialysis prescription was similar in both groups, and the levels of blood urea, serum creatinine, electrolytes, and calcium were comparable between the study groups both at the beginning and at the end of the study period.

DISCUSSION

The factors leading to hearing disorder in uremic patients with ESRF are not exactly defined. One of these multifactorial causes responsible for deterioration of cochlear function seems to be uremic toxins incompletely removed by HD. This assumption is supported by the fact that after successful kidney transplantation and normalization of blood urea and serum creatinine levels, the deafness is reversible.[10-12] Another uremia-induced disorder, polyneuropathy, may also be improved after kidney transplantation. Potentially ototoxic drugs like aminoglycosides, furosemide, ethacrynic acid, or deferoxamine[13-15] may also participate in causing cochlear lesions in uremic patients. None of our study patients received any of these drugs to account for the deafness.

Our results indicate that the degree of renal anemia correlated with the disorder of cochlear function. The only variable factor between the two study groups was the rise in Hb level with EPO treatment, which resulted in improvement of cochlear function in a significant number of patients. Thus, it is reasonable to assume that anemia is an important factor responsible for hearing loss in patients on regular HD. Studies with larger numbers of patients are required to confirm this observation.

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RECONSTRUCTIVE MICROSURGERY IN HEAD AND NECK CANCER

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Ann Otol Rhinol Laryngol 106:1997

HORSERADISH PEROXIDASE PERMEATION FROM THE CAPILLARIES OF THE STRIA VASCULARIS AFTER INOCULATION OF ENDOTOXIN INTO THE MIDDLE EAR

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Escherichia coli–derived endotoxin was inoculated in the middle ear of guinea pigs 24 hours after being injected intraperitoneally. Twenty-four hours after the middle ear inoculation, horseradish peroxidase (HRP) was injected via the femoral vein and the permeability of HRP through the capillaries of the stria vascularis and the destination of the leaked HRP were examined. A large amount of HRP leaked out of the capillary through the opened endothelial cell junctions and penetrated the enlarged intercellular spaces. Leaked HRP entered the pinocytotic vesicles of the intermediate cells. Even slightly degenerated intermediate cells retained this function. The HRP penetrated the spongelike structure of the marginal cells leading to the intercellular space. This structure was not observed without endotoxin. The HRP could not pass to the cochlear duct through the tight junctions between marginal cells. Blood sludging was observed in the strial capillaries. It appeared more frequently in the upper three turns than in the basal turn. The HRP leakage out of the capillaries was observed not only in the upper three turns but also in the basal turn.

KEY WORDS — blood sludging, capillary permeability, electron microscopy, endotoxin, horseradish peroxidase, stria vascularis.

INTRODUCTION

It is known that the capillary permeability of the middle ear mucosa increases after the inoculation of endotoxin.[1,2] Kawauchi et al[3] and Lim et al[4] demonstrated that endotoxin injected into the chinchilla middle ear presented in the inner ear. Pathologic changes of the inner ear were observed after its inoculation.[3-5] The pathologic changes of the stria vascularis previously reported[5] were shrinkage of the intermediate and marginal cells, enlargement of the intercellular spaces, infiltration of the inflammatory cells, and blood sludging in the strial capillaries. But it is unknown whether endotoxin inoculated into the middle ear has an influence on the capillary permeability of the stria vascularis. In this study we intended to clarify the alteration in permeability of the strial capillaries by using horseradish peroxidase (HRP) as a tracer and studying the destination of HRP that has leaked from the capillaries.

MATERIALS AND METHODS

It was clarified in a previous paper[5] that the pathologic changes of the stria vascularis were more severe when endotoxin was intraperitoneally injected before it was inoculated into the middle ear than when it was inoculated without presensitization. Therefore, 10 guinea pigs weighing 350 g were injected intraperitoneally with 0.02 mg of *Escherichia coli*–derived endotoxin (Sigma No. L-2880) in 1 mL of sterile water. Twenty-four hours after the intraperitoneal injection, the animals were anesthetized with pentobarbital (40 mg/kg) and the bullas were opened. A small piece of Gelfoam containing 0.04 mg of endotoxin was placed on the round window. After 24 hours, 15 mg of HRP (Sigma Type VI) dissolved in 1 mL of sterile water was injected via the femoral vein. Five minutes after the injection of HRP, the cochleas were infused with 2% glutaraldehyde through the round window. Five minutes after infusion of the fixative, the animals were decapitated and the cochleas were removed. Each turn of the stria vascularis was microdissected to separate it from the cochlea. These microdissected samples were further fixed for 1 hour with 2% glutaraldehyde and then washed three times with 0.1 mol/L cacodylate buffer (containing 8% sucrose, pH 7.2). They were then incubated for 1 hour at room temperature by a modified Graham-Karnovsky method[6] (0.05 mol/L Tris–hydrochloric acid buffer, pH 7.0, 10 mL containing 0.002% hydrogen peroxide and 8 mg of 3.3 diaminobenzidine tetrahydrochloride). They were then washed three times in 0.1 mol/L cacodylate buffer and postfixed for 40 minutes in 2% osmium tetroxide, dehydrated, and embedded in Epon 812. Ultrathin sections were cut on an LKB ultramicrotome. They were stained with uranyl acetate and lead and examined by transmission electron microscopy. As controls, 3 guinea pigs were used. A small piece of Gelfoam containing physiological saline solution was placed on the round window for 24 hours. After that, the stria vascularis was taken by the method mentioned above.

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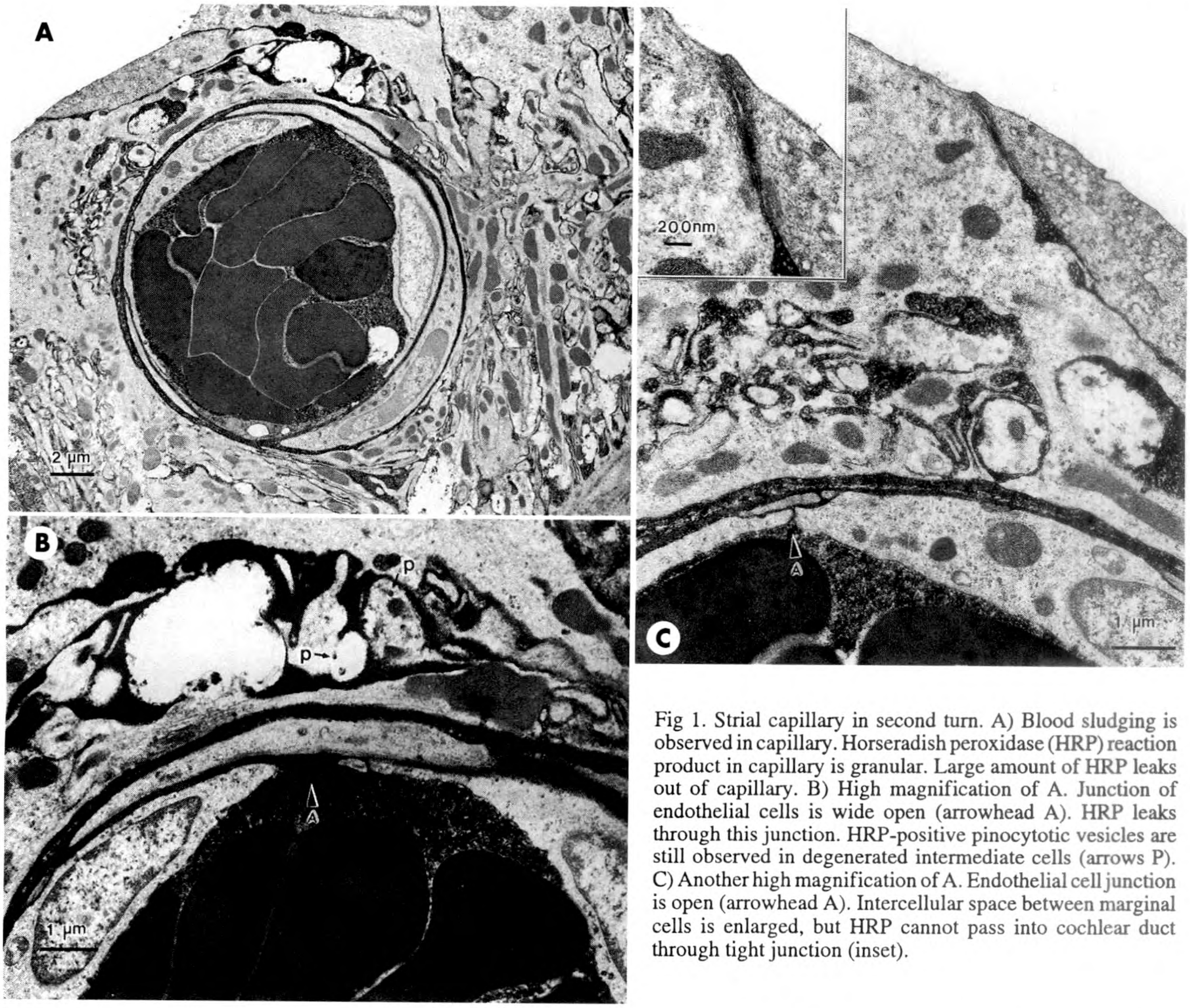

Fig 1. Strial capillary in second turn. A) Blood sludging is observed in capillary. Horseradish peroxidase (HRP) reaction product in capillary is granular. Large amount of HRP leaks out of capillary. B) High magnification of A. Junction of endothelial cells is wide open (arrowhead A). HRP leaks through this junction. HRP-positive pinocytotic vesicles are still observed in degenerated intermediate cells (arrows P). C) Another high magnification of A. Endothelial cell junction is open (arrowhead A). Intercellular space between marginal cells is enlarged, but HRP cannot pass into cochlear duct through tight junction (inset).

RESULTS

Blood sludging was observed in all capillaries of the upper three turns (Fig 1A), but no or slight blood sludging was observed in the basal turn (Fig 2). The HRP reaction product in the capillaries was granular. The HRP leaked out of the capillary in every turn of the cochlea. The intercellular spaces between endothelial cells were wide open (Fig 1B,C, arrowhead A), and large amounts of HRP leaked through the opened endothelial cell junctions. Leaked HRP penetrated into the enlarged intercellular spaces of the stria vascularis (Fig 1A). The HRP, which penetrated between the marginal cells, could not pass into the cochlear duct through the tight junctions (Fig 1C, inset). When the intermediate cells had not degenerated, the HRP reaction product was observed in many of their pinocytotic vesicles (Fig 3A, arrow P). Even if the intermediate cells had degenerated, a small number of HRP-positive vesicles could be observed in them (Fig 1B, arrows P). The HRP reaction product was also observed in the spongelike structure of the marginal cells, which revealed itself by enlarging the intercellular spaces between the complexly inverted cell membranes of the marginal cells (Fig 3A, arrowhead S, and Fig 3B). In control animals, HRP leakage through the capillaries was not observed in every turn of the cochlea (Fig 4).

DISCUSSION

Although Duvall et al[7] showed that HRP was transported across the endothelium of the strial capillaries 10 minutes after intravenous injection, it was shown that HRP did not leak out of the capillaries 10 minutes after the injection, when the animals were decapitated after the cochlear infusion with fixative.[8] In another study, HRP was gradually observed around the capillaries 30 minutes after injection.[9] Large amounts of HRP in our study leaked out of the strial capillaries 5 minutes after the intravenous injection in animals that had been previously inoculated with

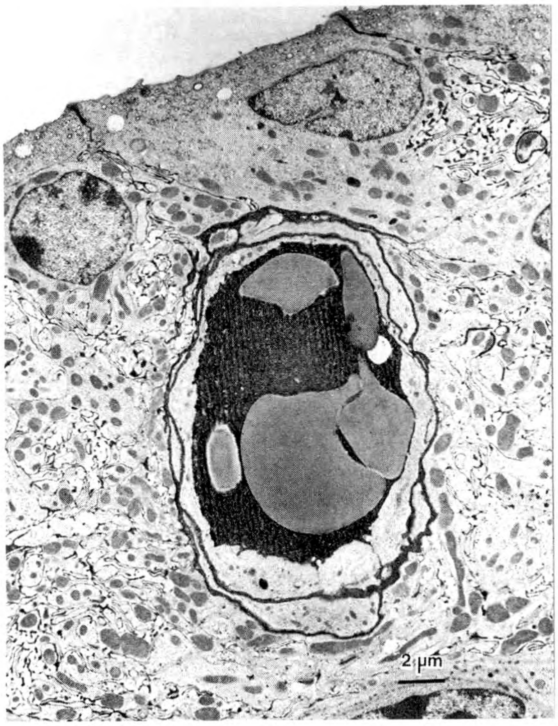

Fig 2. Capillary in basal turn. Although HRP leaks out of capillary, blood sludging is not observed.

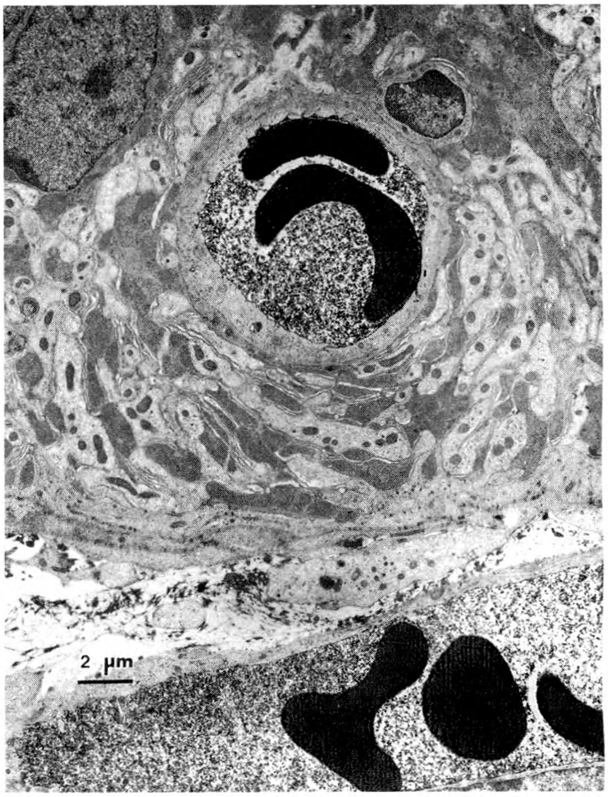

Fig 4. Control. No blood sludging and no HRP leakage is observed in capillaries of stria vascularis or spiral ligament. HRP reaction product in capillary is granular.

endotoxin. It is considered that the reason HRP leaked so quickly was the opening of the junctions of the endothelial cells. Under normal conditions, HRP is transported from inside the capillary to outside it by pinocytotic vesicles, but not through the intercellular junctions of the endothelial cells.[7-10] After the inoculation of endotoxin, the junctions of the endothelial cells opened widely and a large amount of HRP leaked, while a very small number of HRP-positive pinocytotic vesicles were observed in the endothelium. These findings mean that endotoxin accelerated the transportation of HRP not by pinocytosis, but by opening of the endothelial cell junctions. It has previously been reported that the opening of endothelial cell junctions and leakage of erythrocytes into the surrounding tissue are observed in the capillaries of the middle ear mucosa after exposure to endotoxin.[1,2] It was reported that endotoxin promoted the injury of endothelial cells.[11,12] The opening of the endothelial

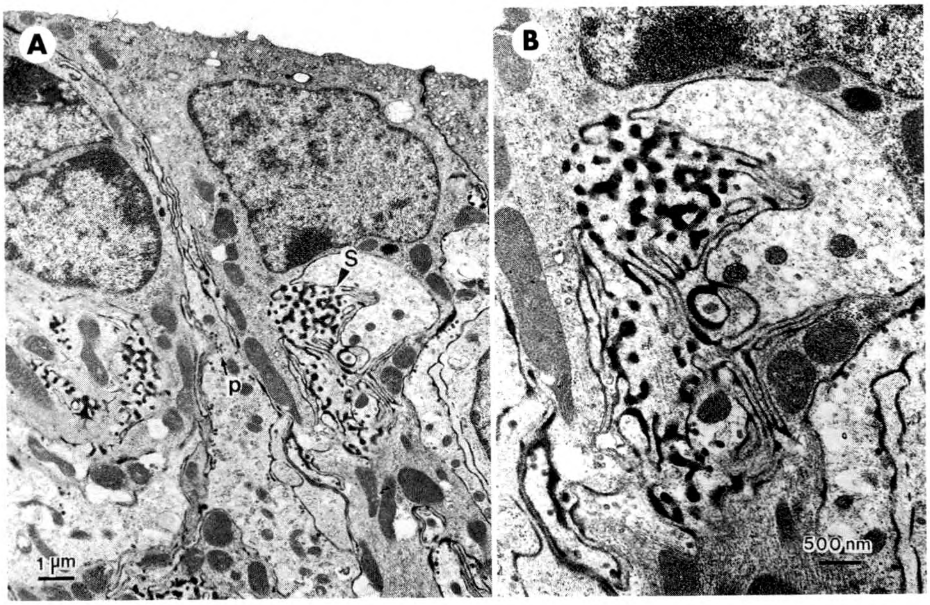

Fig 3. Spongelike structure is observed in marginal cell (arrowhead S). A) Arrow p — pinocytotic vesicles in intermediate cell. B) High magnification of A. Spongelike structure is evident in marginal cell leading to intercellular spaces.

cell junctions is considered to be the first step of the endothelial cell injury caused by the dysfunction of contractile elements in endothelial cells.

The HRP leaking out of the strial capillaries penetrated into the enlarged intercellular spaces of the stria vascularis. The enlarged intercellular spaces, which were also reported in our previous paper,[5] were filled with HRP (Fig 1B). This means that the enlarged intercellular spaces were not an artifact of poor fixation.

Even if the intercellular spaces between marginal cells were enlarged, HRP could not pass through the tight junctions between them. Under normal conditions, HRP in the intercellular spaces is taken into the vesicles of the intermediate cells.[7,9] The pinocytosis of the intermediate cells functioned until the late stage of cell degeneration caused by endotoxin (Fig 1B, arrows P). The HRP also penetrated the intercellular spaces between complexly inverted cell membranes into the marginal cell body, which formed a spongelike structure. In normal stria vascularis, spongelike structures are not observed, as the intercellular spaces do not open and only small amounts of HRP can penetrate between them. As HRP-positive vesicles are not observed in the marginal cells,[7,9] Duvall et al[7] suggested that the intermediate cells might be of the main cell type, taking up substances that pass along the pathway from the vessel to the endolymph. But it was shown in our study that the marginal cells had a dense network of canals leading to the intercellular spaces. These dense networks in the marginal cells may also have an important role in taking up substances that pass along the pathway from the vessel to the endolymph.

No or slight blood sludging was observed in the strial capillaries of the basal turn after exposure to endotoxin, although blood sludging was observed in almost all capillaries of the upper three turns. Even when blood sludging was observed, the HRP reaction product in the capillary was granular (Fig 1A). When animals were decapitated without perilymphatic infusion of fixative, blood sludging appeared in the strial capillaries and the HRP reaction product was not granular but amorphous, because the endothelial cells had been shrunk by the shock of decapitation, and the HRP reaction product in the plasma space that became compressed between the sludged blood cells and the endothelial cells became dense and amorphous.[8] It is considered that the blood sludging observed in this study appeared before HRP injection, as the HRP reaction product was granular. Had blood sludging been caused by the decapitation, the HRP in the capillaries would have been amorphous. The blood sludging in this study, therefore, was not an artifact of decapitation. It is unknown why blood sludging appeared more frequently in the strial capillaries of the upper three turns than in those of the basal turn, notwithstanding the placement of endotoxin on the round window in the basal turn. Blood sludging in the strial capillaries is also observed in severe hemorrhagic shock.[13] It is interesting to note that in both this study and the previous study, blood sludging was frequently observed in the upper three turns.

In this study, the stria vascularis was taken 24 hours after the inoculation of endotoxin in the middle ear. Kawauchi et al[3] reported that endotoxin in the perilymph peaked at 24 to 48 hours after injection in the middle ear. In our previous paper,[5] the pathologic changes of the stria vascularis, including the shrinking of marginal and intermediate cells, enlargement of the intercellular spaces, and blood sludging in the strial capillaries, were obvious at 24 hours postinoculation. In this study, therefore, the stria vascularis was taken 24 hours after the inoculation.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria (in English July 3-5, 1997, and in German September 4-6, 1997). For more information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax 43/316/385/3425.

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COMPARISON OF SOFT TISSUE RESPONSE IN RABBITS FOLLOWING LARYNGEAL IMPLANTATION WITH HYDROXYLAPATITE, SILICONE RUBBER, AND TEFLON

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This study evaluates the soft tissue response in rabbits following laryngeal implantation for medialization using hydroxylapatite prostheses, carved silicone rubber prostheses, and injectable Teflon. Sixteen rabbits underwent left recurrent laryngeal nerve section for denervation and laryngeal implantation with hydroxylapatite. At 1, 3, 6, and 12 months, 4 animals were painlessly sacrificed and processed for histology. Similarly, animals were implanted with carved silicone rubber prostheses or with Teflon injected through a flap in the thyroid lamina for comparison at 1, 3, and 6 months. In animals implanted with hydroxylapatite, histologic findings include limited acute inflammatory response, thin fibrous encapsulation, and osteogenesis in the region of the fenestra, with lamellar bone bridging the space between the implant and thyroid lamina. With silicone rubber prostheses, there is a limited inflammatory response and fibrous encapsulation of the implant without evidence of osteogenesis. Animals implanted with Teflon demonstrated a classic foreign body reaction with multinucleated giant cells, granuloma formation, and migration of Teflon into surrounding muscle. With respect to soft tissue response, both hydroxylapatite and silicone rubber are less reactive than Teflon. The osteogenesis observed in the presence of hydroxylapatite increases implant stability and minimizes the risk of migration. Conversely, the presence of bone growth may limit the reversibility of medialization procedures performed with hydroxylapatite.

KEY WORDS — histology, hydroxylapatite, laryngeal implants, silicone rubber, Teflon, thyroplasty, vocal fold paralysis.

INTRODUCTION

Vocal fold paralysis or paresis results in an impaired ability to phonate and is frequently associated with various degrees of dysphagia and aspiration. Management of this problem includes medialization of the affected fold by vocal fold injection, thyroplasty, or reinnervation of the paralyzed adductor muscles. Both injection and thyroplasty require implant material to maintain the vocal fold in an adducted position. Aside from autologous tissue (fat or cartilage), the only material approved specifically for vocal fold medialization is Teflon. Silicone rubber block (dimethylpolysiloxane) is used routinely for medialization thyroplasty; however, silicone rubber has not been approved specifically for vocal fold medialization. The authors have recently reported clinical experience with prefabricated hydroxylapatite implants for medialization thyroplasty.[1] This study is designed specifically to assess the soft tissue responses following laryngeal implantation with Teflon, silicone rubber, and hydroxylapatite for vocal fold medialization.

METHODS

Medialization With Hydroxylapatite. New Zealand white rabbits (16) were sedated with an intramuscular combination of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Supplemental doses of ketamine were administered intravenously at 30-minute intervals as needed to maintain adequate anesthesia. The anterior neck was shaved, prepared with Betadine, and draped for surgery with the head in the neutral position. A transverse incision was made at the level of the midthyroid ala. The midline raphe of the strap muscles was incised and the strap muscles were reflected laterally. The recurrent laryngeal nerve was sectioned on the side to be implanted. A window was then created in the thyroid cartilage to accommodate the prosthesis. The upper horizontal aspect of the window is made at the level of the mid-ala and centered in the anteroposterior direction. The cartilage was removed and the inner perichondrium left intact. (As a technical note, the inner perichondrium appears as a thin, wispy membrane, and at the time of surgery, preser-

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Presented at the meeting of the American Laryngological Association, Palm Beach, Florida, May 7-8, 1994.

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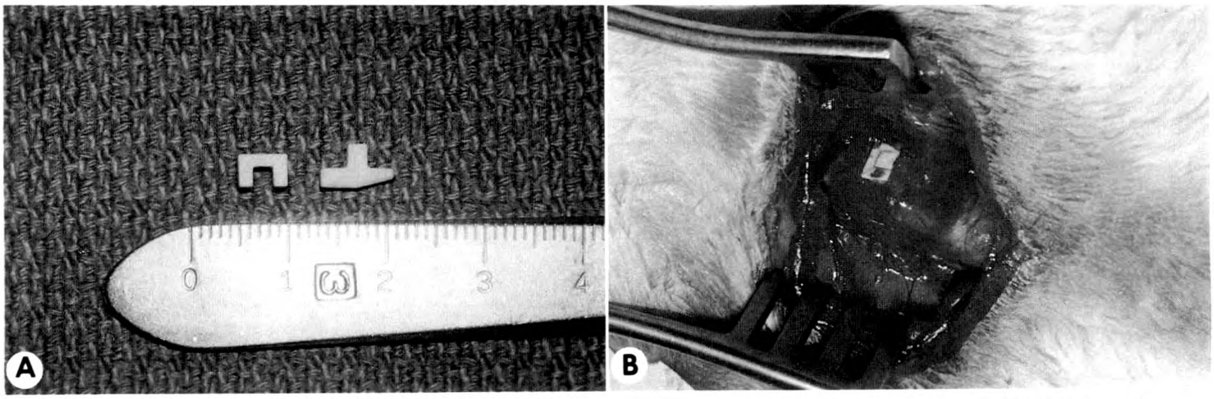

Fig 1. Hydroxylapatite implants used for implantation in rabbit larynges. A) Two implants shown with locking shim, similar to implants described previously by Cummings et al.[1] B) Implant inserted in rabbit larynx at level of vocal folds with locking shim in place.

vation of this layer is difficult to document. Moreover, if the inner perichondrium remains attached to the portion of cartilage removed, then medialization of this layer would not be accomplished. In this condition the implant would interface with laryngeal muscle without an intervening layer of perichondrium.) A preformed hydroxylapatite implant (Smith & Nephew Richards, Inc) was inserted and secured with a stabilizing shim (Fig 1[1]). The wound was then closed with a running 4-0 suture. During the procedure, the animal's temperature was maintained with a thermostatically controlled heating pad.

At 1, 3, 6, and 12 months, 4 animals were euthanized and their larynges fixed and examined histologically. Whole larynges were fixed in formalin, decalcified, and imbedded in paraffin. Whole transverse sections were then cut at 25 μm, mounted on glass slides, and stained with hematoxylin and eosin (H & E). Representative sections at the implant level were also stained with trichrome for demonstration of connective tissue.

Implantation With Silicone Rubber. New Zealand white rabbits (9) were implanted with silicone rubber implants by the same anesthesia and surgical techniques outlined above. Silicone rubber implants, similar to the hydroxylapatite implants, were carved from firm silicone rubber block (Dow Corning). Three animals at 1, 3, and 6 months were euthanized and their larynges fixed and examined histologically.

Implantation With Teflon. New Zealand white rabbits (9) were injected with Teflon paste (Mentor Inc) with the same anesthesia outlined for implantation with hydroxylapatite. The surgical approach was altered in this group. Instead of creating an open fenestra, we created a U-shaped thyroid cartilage flap at the level of the vocal fold. Teflon paste (0.1 mL) was injected into the paraglottic space adjacent to the thyroarytenoid muscle, the flap returned to its anatomic position, and the wound closed. Three animals at 1, 3, and 6 months were euthanized and their larynges harvested and examined histologically.

Control Animals. New Zealand white rabbits (4) were anesthetized and a laryngeal fenestra was created by the surgical technique outlined for implantation with hydroxylapatite and silicone rubber. Two animals were euthanized at 1 and 3 months and their larynges were fixed and examined histologically.

RESULTS

Histologic Changes Observed in Control Animals. In 4 animals, the recurrent laryngeal nerve was sectioned and a fenestra created. No implant was placed in these animals. Two animals were sacrificed at 1 and 6 months. After 1 month, there were myxomatous changes with associated muscle degeneration. There was no inflammatory response. The mucosa on the denervated side was mildly thickened with increased cellularity of the mucosal layer (acanthosis). Salivary gland acini were normal in appearance. After 6 months there was evidence of reinnervation and the ipsilateral vocal fold mucosa and salivary gland acini were normal in appearance.

Histologic Changes Observed Following Hydroxylapatite Laryngeal Implantation. Four animals were euthanized at 1 month, and the larynges were harvested and prepared for histology. With H & E staining, in all 4 animals, the implants were decalcified and only small fragments of proteinaceous material were observed in the periphery. A thin, uniform condensed fibrous layer surrounding the implant and shim with adjacent histiocytic reaction and rare foreign body giant cells was seen. There were scattered eosinophils, lymphocytes, and plasma cells in the surrounding stroma. Few segmented neutrophils were seen. There were myxomatous changes and associated muscle degeneration on the denervated side.

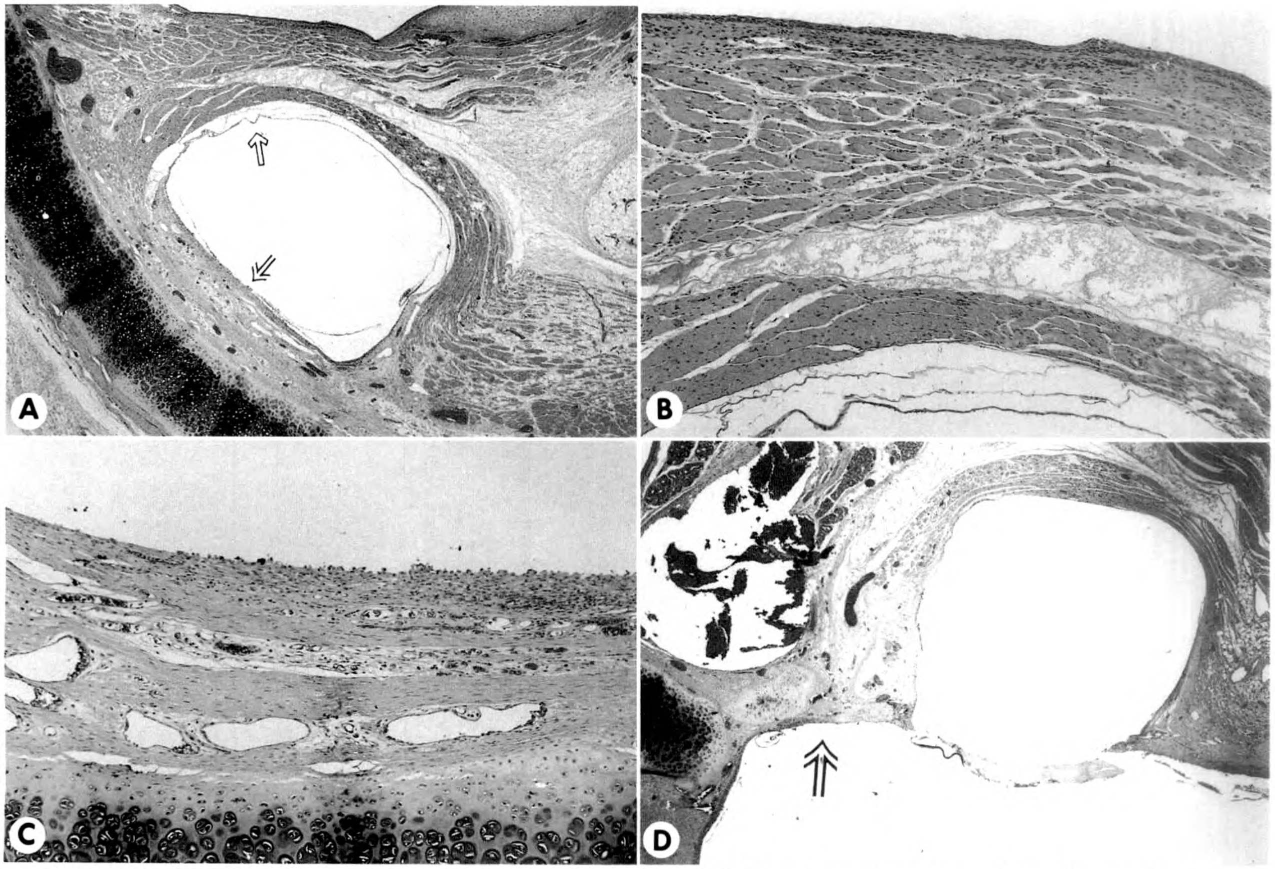

Fig 2. Rabbit larynx 1 month after placement of hydroxylapatite implant. A) There is pronounced muscle atrophy associated with denervation (original ×25). There is limited inflammatory response and thin fibrous capsule surrounding implant. B) Higher magnification demonstrating implant-muscle interface (closed arrow in A; original ×100). There is thin fibrous capsule that has separated from overlying muscle. There is minimal inflammatory component consisting predominantly of lymphocytes and occasional eosinophils and segmented neutrophils. C) Interface between implant and thyroid cartilage (open arrow in A) demonstrating angiogenesis, moderate inflammatory response consisting of lymphocytes, plasma cells, and eosinophils, and intervening fibrous tissue (original ×100). D) Region of fenestra demonstrating early hyaline cartilage formation (arrow).

Early focal hyaline cartilage formation was identified in the region of the fenestra only, adjacent to the implant and shim (Fig 2). The epithelium of the vocal fold ipsilaterally was thickened and acanthotic in 2 of 4 animals.

At 3 months, 4 animals were sacrificed and larynges harvested. Specimens were processed as described above. With H & E staining, in all 4 animals, the implants were decalcified. There was a thin, uniform condensed fibrous layer surrounding the implant and shim. The fibrous reaction was more pronounced laterally between the implant and thyroid cartilage. New bone was forming in the thyroid lamina fenestra and had completely filled the fenestra in 1 animal. Evidence of denervation, including myxomatous changes within the connective tissue and associated muscle degeneration, was seen on the side of the implant. Acanthosis, or thickening of the epithelium, was seen on the implanted side, adjacent to the implant. This was unchanged as compared to 1-month animals. Plasma cells and eosinophils were abundant in the stroma surrounding the implant. Occasional segmented neutrophils and lymphocytes were also seen. Focal areas of histiocytes were present.

Trichrome staining in the 1- and 3-month animals (not shown) was used to demonstrate connective tissue elements. A thin, uniform fibrous layer was observed surrounding the implants.

At 6 months, 4 animals were sacrificed and their larynges harvested. Specimens were processed for H & E and trichrome stains. With H & E staining, in all 4 animals, there was evidence of reinnervation, the space occupied by the implant was easily identified, and the muscle was compressed and medialized. There were focal areas of acute inflammation with lymphocytes, plasma cells, and eosinophils (Fig 3A,B). The thin fibrous encapsulation of the implant had not changed as compared to 1-month and 3-month animals. Scattered collections of histiocytes were seen surrounding the implant and there was prominent fibrosis laterally. There was lamellar bone in the fenestra bridging the thyroid lamina and hydroxylapatite shim. No bone or cartilage formation

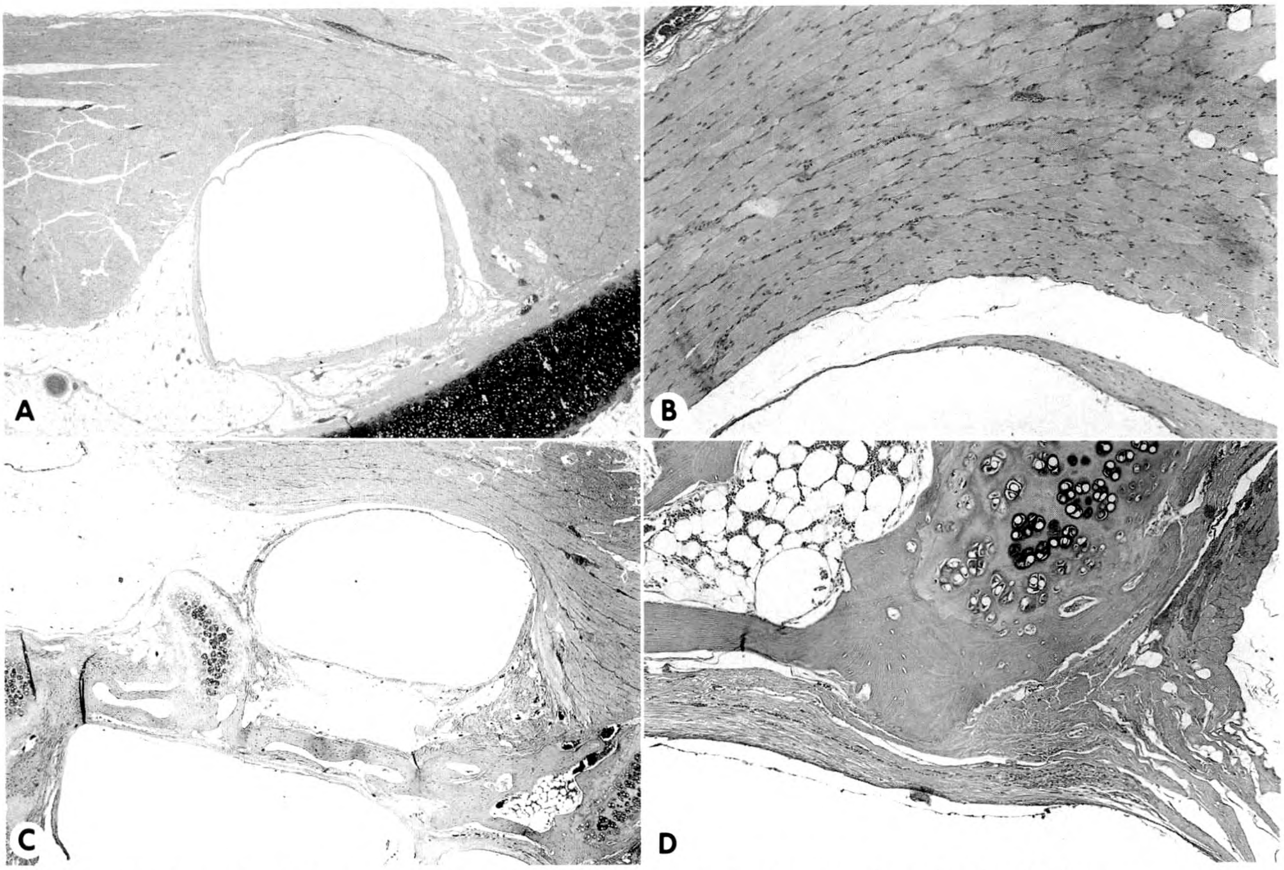

Fig 3. Rabbit larynx 6 and 12 months after placement of hydroxylapatite implant. A) At 6 months, there is fibrous encapsulation of implant and histiocytes adjacent to implant consistent with foreign body reaction (original ×25). There is evidence of muscle reinnervation. B) Muscle-implant interface at 6 months, showing fibrous encapsulation with limited histiocytic reaction in lower right (original ×200). There is no evidence of acute inflammatory response. There is evidence of muscle reinnervation. C) Larynx 12 months after implantation with hydroxylapatite (original ×25). There is fibrous encapsulation of implant. Bridge of mature lamellar bone has grown through fenestra and is seen separating locking shim and implant. Focus of cartilage growth is also seen. Again, there is no evidence of chronic inflammation or foreign body reaction. D) Higher magnification of fenestra at 12 months, showing mature lamellar bone bridging fenestra and focus of cartilage growth. Fibrous tissue is surrounding implant with limited cellular response.

was seen medially, either adjacent to the implant or within the soft tissue of the vocal fold. The acanthosis of the mucosal membrane observed in 1- and 3-month animals was less pronounced in this group and seen only in 2 specimens. The salivary glandular material that was present was normal in appearance.

With trichrome in the 6-month animals, as seen in 1- and 3-month animals, the connective tissue was stained, demonstrating a thin, uniform fibrous layer surrounding the implants. Medially, the capsule, at its thickest region, was only a few cell layers thick, while prominent fibrosis was seen lateral to the implant, adjacent to the regions of osteogenesis.

At 12 months, 3 animals were sacrificed and their larynges harvested. One animal died of unknown causes in the postoperative period. Specimens were processed for H & E and trichrome stains. With H & E staining, there was evidence of reinnervation in all 3 animals. There was a thin fibrous encapsulation of the implant, which appeared unchanged when compared to 1-, 3-, and 6-month animals. Occasional collections of histiocytes were seen adjacent to the implant. Overall, there was a pronounced decrease in inflammatory cells and no evidence of giant cell formation. There was dense, mature lamellar bone in the fenestra bridging the thyroid lamina and hydroxylapatite shim. There was no bone or cartilage formation medially adjacent to soft tissue (Fig 3C,D).

With trichrome stain in the 12-month animals, the thin, uniform fibrous layer surrounding the implant was unchanged from that in 1-, 3-, and 6-month animals.

Histologic Changes Observed Following Silicone Rubber Laryngeal Implantation. At 1 month, 3 animals were sacrificed and larynges harvested. Specimens were processed for H & E and trichrome stains. With H & E staining, the implant was partially dissolved during tissue processing. There was a thin,

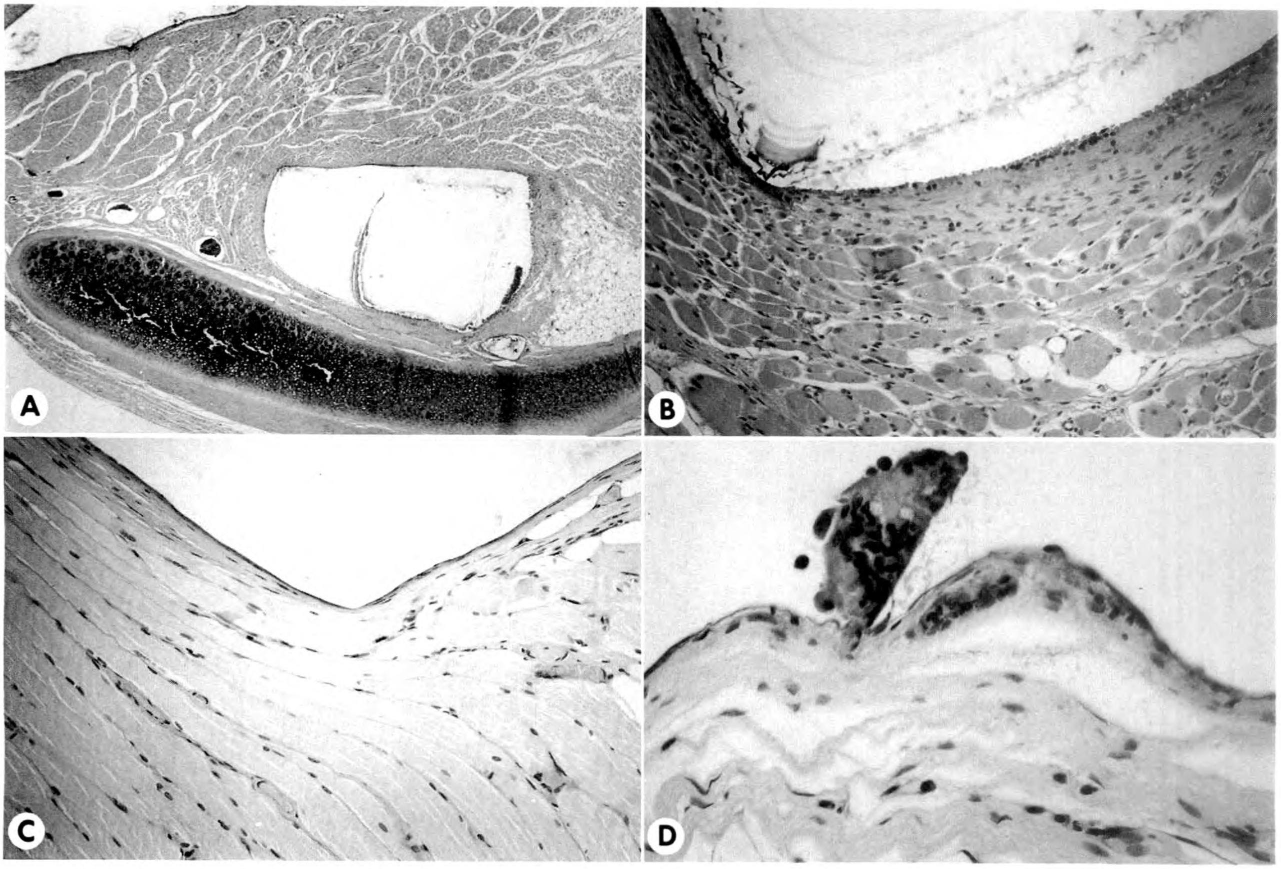

Fig 4. Rabbit larynx after implantation with silicone rubber prosthesis. A) At 1 month, implant is medial to thyroid cartilage and lateral to thyroarytenoid muscle. There is evidence of denervation with muscle atrophy. B) High-power view of muscle-implant interface at 1 month, demonstrating cellular infiltrate with plasma cells, lymphocytes, eosinophils, and histiocytes (original ×200). C) Muscle-implant interface in rabbit larynx 6 months after placement of silicone rubber implant (original ×200). There is thin fibrous layer surrounding implant with absence of inflammatory component. D) Same animal with histiocytic response, including foreign body giant cell (original ×400). Plasma cells can also be seen within adjacent stroma.

uniform condensed fibrous layer surrounding the implant. Plasma cells, lymphocytes, eosinophils, and segmented neutrophils were seen in the surrounding stroma (Fig 4A,B). Myxomatous changes and associated muscle degeneration were present on the denervated side. There was no evidence of hyaline cartilage formation in the vicinity of the fenestra. The epithelium of the ipsilateral vocal fold was thickened and acanthotic. There were no chronic inflammatory changes identified.

With trichrome staining in the 1-month animals, as seen in hydroxylapatite animals, there was a thin, uniform fibrous layer surrounding the implant.

At 3 months, 1 animal had died of unknown causes in the postoperative period, so 2 animals were euthanized and their larynges were harvested. With H & E staining, there was a thin, uniform condensed fibrous layer surrounding the implant and shim. The degree of fibrosis was less as compared to that in hydroxylapatite animals. Collections of lymphocytes, plasma cells, eosinophils, and segmented neutrophils were seen in the surrounding stroma. Histiocytes and occasional giant cells were seen. Acanthosis or thickening of the epithelium was seen in the implant side, adjacent to the implant.

At 6 months, 3 animals were sacrificed and their larynges harvested. Evidence of muscle reinnervation was present. There was a persistent thin fibrous encapsulation of the implant without a change in thickness as compared to 1- and 3-month animals. Scattered lymphocytes, plasma cells, and eosinophils were seen in the surrounding stroma, and small collections of histiocytes were seen adjacent to the implant with rare giant cell formation (Fig 4C,D). There was no evidence of osteogenesis or cartilage formation in the area of the fenestra. The ipsilateral vocal fold was normal in appearance.

Histologic Changes Observed Following Teflon Laryngeal Implantation. At 1 month, 3 animals were sacrificed and their larynges harvested. On the side of Teflon implantation, there was a mass of intense foreign body reaction with lymphocytic infiltrate, eosinophils, and foreign body giant cells. There was increased vascularity with angiogenesis. Teflon par-

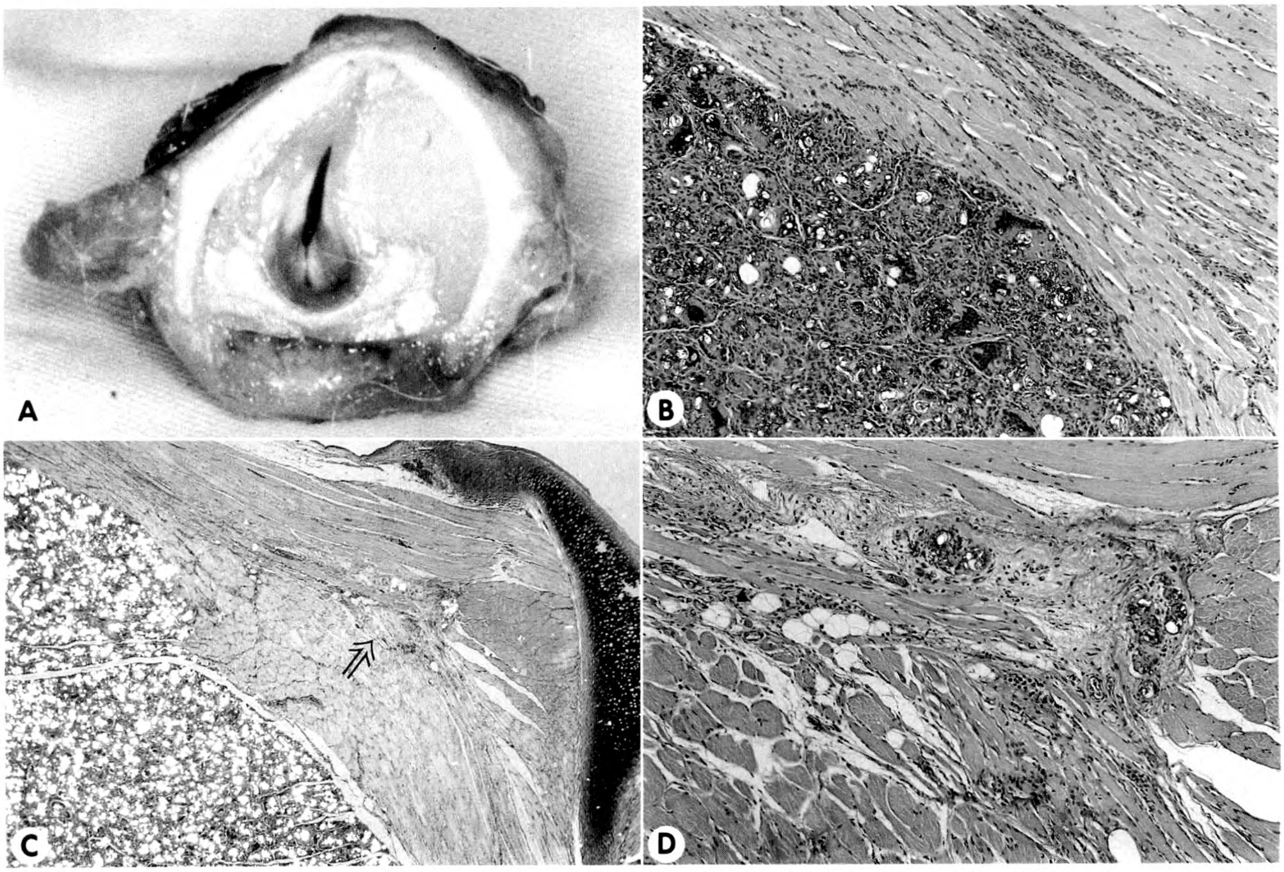

Fig 5. Rabbit larynx 6 months following Teflon injection. A) Whole cross section at level of vocal folds. Large mass has filled paraglottic space and displaced vocal fold beyond midline. B) Photomicrograph of larynx demonstrating Teflon granuloma with multinucleated giant cells surrounding refractile Teflon particles (original ×200). Adjacent muscle demonstrates infiltration by lymphocytes and eosinophils. C) Low-power view of Teflon granuloma at vocal process (original ×25). Refractile bodies representing Teflon particles are seen within granuloma. Refractile bodies are also seen within thyroarytenoid musculature. Arrow — area enlarged in D. D) Teflon particles, having migrated into thyroarytenoid muscle, are surrounded by multinucleated giant cells (original ×200). Surrounding muscle is infiltrated by lymphocytes and eosinophils.

ticles were seen as refractile bodies dispersed throughout the soft tissue mass surrounded by histiocytes. Refractile Teflon particles were also seen within the adjacent muscle body. There were myxomatous changes and associated muscle degeneration on the ipsilateral side. There was no evidence of cartilage or bone formation in the region of the fenestra. Acanthosis of the ipsilateral vocal fold was observed.

At 3 months, 3 animals were sacrificed and their larynges harvested. In all 3 animals, there was a persistent foreign body reaction with infiltration of lymphocytes, eosinophils, and foreign body giant cells as seen in the 1-month animals.

At 6 months, 3 animals were sacrificed and their larynges harvested. In all animals, there was a persistent foreign body giant cell reaction. In 2 of 3 animals, there was a clear progression of granuloma formation and an obvious mass effect. Teflon particles had migrated medially into the muscle body, associated with intense lymphocytic infiltrate and giant cell formation (Fig 5). Reinnervation had occurred and the vocal fold mucosal layer was normal in appearance.

DISCUSSION

Selection of implant material for clinical use should be based on specific criteria. In general, implant material should be 1) readily available, 2) easily applied and contoured, 3) positionally stable, 4) resistant to degradation, 5) effective without injury to surrounding tissue, 6) immunologically inert, and 7) noncarcinogenic. Of the available materials used today for vocal fold medialization, Teflon meets the fewest criteria. Teflon is readily available and easily applied by injection; however, overinjection and misplaced Teflon injections may result in a mass effect and subglottic or intracordal injections.[2,3] The mechanical effects of overinjection or misplaced injections disrupt normal airflow and vocal fold vibration. Teflon has also been reported to migrate and present in extralaryngeal sites.[4] As shown in this study, the characteristic histologic response to Teflon is a nonspecific foreign body reaction. There is infiltration by lymphocytes, eosinophils, and histiocytes with giant cell formation. The Teflon particles are dispersed throughout the granuloma surrounded by giant cells. Migration of Teflon particles into adjacent muscle results in a diffuse inflammatory response as well as localized giant cell formation. The diffuse intramuscular inflammatory response correlates with clinical findings, specifically, impaired vibratory characteristics following injection with Teflon.[5,6] The foreign body reaction is typically self-limiting; however, a mass effect due to granuloma formation long after injection may result in progressive dysphonia and/or airway obstruction. The immunologic response to Teflon particles may be limited to cellular responses, and there is no known association with autoimmune disorders. Cultured human monocytes in the presence of expanded polytetrafluororethylene (e-PTFE), used for vascular grafts, express both tumor necrosis factor and interleukin-1.[7] Positive leukocyte adherence inhibition tests (a measure of T cell reactivity) have also been demonstrated in patients with e-PTFE fabric vascular grafts.[8]

Medical-grade silicone, or Silastic (Dow Corning), has been readily available, although recent problems implicating silicone breast implants in development of acquired autoimmune diseases may curtail the availability of medical-grade silicone. Silicone rubber is easily applied for vocal fold medialization. Migration of implants into the airway has been reported and may represent technical errors and not rejection of a foreign body.[9] Silicone rubber has been considered relatively inert in biological systems and resists degradation; however, a foreign body reaction with giant cell formation does occur in patients implanted with rubberized silicone.[10] Mild deterioration of medical-grade silicone rubber has also been demonstrated microscopically in voice prostheses.[11] In this setting, degradation is thought to be secondary to mechanical, chemical, or enzymatic degradation by filamentous and vegetative yeast cells. Degradation of hard silicone may also occur in a sterile environment, as demonstrated histologically with breast implants.[12] Enzymatic digestion by macrophages is one postulated mechanism. Silicone-to-silica conversion by macrophages producing superoxide free radicals has also been proposed.[13]

Although autoimmune disorders have not been demonstrated with implantation of solid silicone implants, both cellular and humoral responses are known to occur.[14,15] Macrophages are found in high concentrations in tissue injected with free silicone or implanted with solid implants.[16] A foreign body reaction to silicone has been reported following free silicone injections, breast implants, and implantation of joint prostheses.[12,16] Joint prostheses are associated with synovial cell hyperplasia and vascular proliferation.[15,17] Synovitis with mononuclear cell aggregates and giant cell reactions occurs with silicone joint replacement, and distal granulomas may present in regional lymph nodes.

Humoral responses have been demonstrated experimentally and clinically after implantation with silicone. Inhibition of macrophage migration and lymphocyte production of macrophage-inhibiting factor occurs in animals injected with silicone oil–Freund's adjuvant mixture.[13] In this situation delayed-type hypersensitivity responses occur in sensitized animals.[18] Sensitization with silicone carpal prosthesis fragments and Freund's adjuvant also results in cellular and humoral responses.[19] Clinically, antibody production (IgG) to elastomers of polydimethylsiloxane has been demonstrated in two patients with reactions to ventriculoperitoneal shunts.[20] Similarly, antibody production has been shown to occur in women implanted with silicone breast implants.[13]

Although cellular and humoral responses occur with exposure to silicone or silicone-Freund mixture, a causal relationship to autoimmune diseases has not been established.[15] Furthermore, there have been no reported cases of or associations with autoimmune disease after implantation with joint prostheses.

Hydroxylapatite is a bioactive polycrystalline ceramic composed of calcium and phosphate.[21] Hydroxylapatite has gained favorable attention for use as a biocompatible orthopedic, dental, and otologic implant material. Two forms of hydroxylapatite are available: porous and dense. Dense hydroxylapatite is relatively inert and resists degradation at the implant-tissue interface. Direct chemical bonding between bone and hydroxylapatite does occur. The soft tissue response to hydroxylapatite can be either direct cell growth into the surface of hydroxylapatite or encapsulation, with a thin fibrous layer surrounding the implant.[22,23] In general, the material is nonreactive and immunologically inert. It is not easily sculpted, and prefabricated implants are required.

Following laryngeal implantation (hydroxylapatite), there is an acute inflammatory response with scattered lymphocytes, plasma cells, eosinophils, and occasional segmented neutrophils appearing in the stroma surrounding the implant. Between 1 and 6 months, in the interface between soft tissue and hydroxylapatite, there is a thin fibrous capsule adherent to the hydroxylapatite and focal areas of histiocytes. In the region of the fenestra where hydroxylapatite closely approximates cartilage, new cartilage formation is seen. Chronically, cartilage is replaced with bone formation between the fenestra and hydroxylapatite. At 12 months, there is no inflammation in the vicinity of the hydroxylapatite implant, and the soft tissue response is limited to a thin fibrous capsule adjacent to muscle and thin layers of histiocytes adjacent to fibrous tissue. Osteogenesis with lamellar bone formation occurs at the thyroid lamina fenestra and bridges the space between the thyroid lamina and the hydroxylapatite implant and shim. Other findings, including myxomatous changes associated with muscle degeneration and acanthosis or thickening of the epithelium, appear to be related to denervation and are absent in animals surviving 6 to 12 months.

The absence of chondrogenesis or osteogenesis at the interface between soft tissue and hydroxylapatite suggests a difference in the bioactive response of hydroxylapatite at the soft tissue interface when compared to activity at the cartilage implant interface. This may be the result of an altered molecular environment where soft tissue interfaces with the implant or, alternatively, the result of surgical removal of the inner perichondrium, thus removing the osteogenic potential of the surrounding tissue.

The relevant implications of the soft tissue response to hydroxylapatite for vocal fold medialization are related to the stability of the implant and the reversibility of the procedure. Osteogenesis associated with hydroxylapatite seen in the region of the fenestra theoretically will result in implant stabilization and limit the potential for migration or slippage of the implant. Conversely, this bone growth will obliterate tissue planes between the hydroxylapatite implant and soft tissue, creating difficulty during attempts to remove the implant for revision. Our clinical experience supports this assumption, and removal of hydroxylapatite implants requires sharp dissection along the tissue-implant interface and removal of hydroxylapatite with a drill and diamond bur. Removal, however, may be accomplished with minimal trauma to adjacent tissue. Stabilization of silicone rubber implants is determined solely by surgical technique. In the absence of osteogenesis and bonding between silicone and soft tissue, silicone rubber is more easily removed. In contrast, Teflon particles become dispersed throughout the granulomatous reaction, migrate into adjacent muscle, and incite an intense inflammatory response. As a result, Teflon is extremely difficult to remove, and Teflon injection should not be considered a reversible procedure.

CONCLUSIONS

In our comparison of the tissue response to various implant materials for vocal fold medialization, Teflon was the least desirable material and met the fewest criteria for an acceptable implant material. Silicone rubber is easily applied for medialization thyroplasty, although future availability is questionable. Mechanical fixation or chemical bonding to surrounding structures does not occur with silicone rubber, and unless the implant is adequately stabilized or fixed to surrounding structures, migration remains a potential problem throughout the patient's life. Hydroxylapatite meets all criteria for an acceptable implant material. The bioactive nature of hydroxylapatite results in fixation of the implant to surrounding structures and theoretically minimizes the potential for slippage or migration. The disadvantages of hydroxylapatite include difficulty in modifying or reshaping the implant and difficulty in removing the implant due to bonding between the implant and soft tissue.

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INVESTIGATION OF THE 4,000-HERTZ DIP BY DETAILED AUDIOMETRY

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It is well known that a 4,000-Hz dip is often observed in the initial stage of noise-induced hearing loss and acoustic trauma, but it does not always occur as a result of these causes, and quite often the cause is unknown. Pure tone audiograms of 159 patients (76 cases of noise-induced hearing loss, 62 cases of hearing loss of unknown cause, 21 cases of familial hearing loss) with a 4,000-Hz dip were studied by detailed audiometry to clarify this configuration. The types of dip were classified into 11 groups; however, the basic types are the cone type, bowl type, and dish type, which show the smooth line of the hearing threshold. There were no clear differences in dip configuration among the 3 causes of hearing loss. It was thought that the dips displayed similar configurations regardless of the cause.

KEY WORDS — detailed audiometry, dip audiogram, 4,000-Hz dip.

INTRODUCTION

In general, when hearing loss is localized at 4,000 Hz and observed on an octave audiogram, it is referred to as the 4,000-Hz dip or the c5 dip. (More accurately, c5 is 4,096 Hz, so strictly speaking, the 4,000-Hz dip and the c5 dip are not the same.) This hearing loss is often observed in the initial stage of noise-induced hearing loss and acoustic trauma, but it does not always occur as a result of these causes, and quite often the cause is unknown.

The pure tone audiometry routinely performed in ordinary clinical practice is octave audiometry, in which hearing thresholds are measured at seven frequencies: 125, 250, 500, 1,000, 2,000, 4,000, and 8,000 Hz. Since no other frequencies are tested, however, it is impossible to determine thresholds except at the frequencies listed above. The pure tone audiograms that exhibit dips at 4,000 Hz give the impression that hearing loss occurs only at 4,000 Hz, but there is a strong suspicion that the damage of the dip is not wedge-shaped and localized at 4,000 Hz, but is centered at 4,000 Hz and extends to surrounding frequencies. Nevertheless, questions such as how far the damage extends beyond 4,000 Hz, and whether the damage pattern conforms to the wedge shape seen on the octave audiogram, have not yet been intensively studied.

In this study, I carefully assessed the 4,000-Hz dip using the detailed audiometry devised by Tsuiki et al.[1] Hearing threshold is more precisely recorded than it would be by using the Bekesy audiometry conditioned method.

SUBJECTS

The subjects of this study were 159 patients whose pure tone audiogram revealed a dip at 4,000 Hz, in whom the 2,000- and 8,000-Hz hearing levels were within 20 dB, and in whom the dip was at least more than 20 dB from their threshold at 2,000 or 8,000 Hz, whichever was higher. The subjects, 147 males and 12 females ranging from 8 to 69 years of age (mean 36.9 years), were classified according to the cause of their hearing loss as follows: 1) noise-induced (76 cases), 2) no clear cause identified (62 cases), and 3) familial (21 cases). Familial hearing loss was judged to be present if either or both of the patient's parents had sensorineural hearing loss similar to the patient's, or resembling it in the high-frequency range in a pure tone audiogram.

It is possible to analyze various types of dip in pure tone audiograms. Therefore, in this report, the dips mentioned in the above conditions were analyzed. These types of dip are easy to understand and characterize by their form. Other types of dips will require study in the near future.

METHODS

I measured hearing thresholds of the 2 octaves from 2,000 to 8,000 Hz using the detailed audiometry method devised by Tsuiki et al.[1] The principle of detailed audiometry is similar to that of Bekesy audiometry, but with the following changes in the measurement parameters: 1) rate of frequency change, 5 minutes 40 seconds per octave, 2) rate of intensity change, 2 dB per second, 3) rate of paper speed, 8.5 cm per octave, and 4) stimulus tone, pure tone repetition period, 500 milliseconds, and duty cycle, 50%.

The measurement limit for hearing threshold changes due to frequency changes under the above conditions is theoretically 680 dB per octave. This

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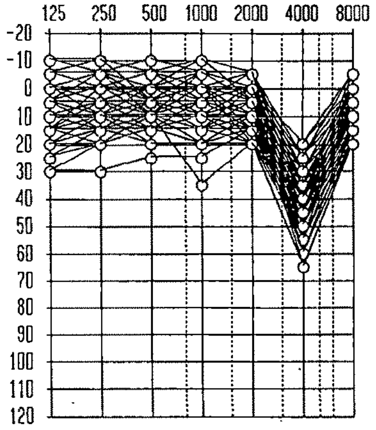

Fig 1. Superimposed audiograms of all patients.

means that the angle at the threshold of the beginning frequency of the dip and the threshold curve of the dip (sloping angle of the descending part of the threshold curve) can be examined at the level of about 80° in a detailed audiogram. Therefore, under these recording conditions, it is expected that the threshold at all frequencies can be correctly recorded in almost all dip cases.

RESULTS

Configuration of Dips on Pure Tone Audiograms. The superimposed audiograms of the 159 subjects who took part in this study are shown in Fig 1. Their mean hearing levels at 2,000, 4,000, and 8,000 Hz were 6.5, 39.9, and 9.0 dB, respectively, with lowest and highest hearing levels at 4,000 Hz being 20 dB and 65 dB, respectively.

Types of 4,000-Hz Dip Observed on Detailed Audiograms. The types of 4,000-Hz dip on the detailed audiograms were classified into the following 11 main groups, which include the 3 basic types (A-C):

A — Cone type (Fig 2).

B — Bowl type (Fig 2).

C — Dish type (Fig 2).

D — The curve comes downward, forms a small dip (microdip) or notch, then becomes horizontal and gradually goes upward. Cases in which the microdip is seen in the left portion (low frequency range) of the horizontal part of the dish type (Fig 3).

E — Cases in which the microdip is observed almost in the center of the horizontal part of the dish type (Fig 3).

F — Cases in which the microdip is seen in the right portion (high frequency range) of the horizontal part of the dish type (Fig 3).

G — Cases in which a few microdips are observed in the downward curve (Fig 4).

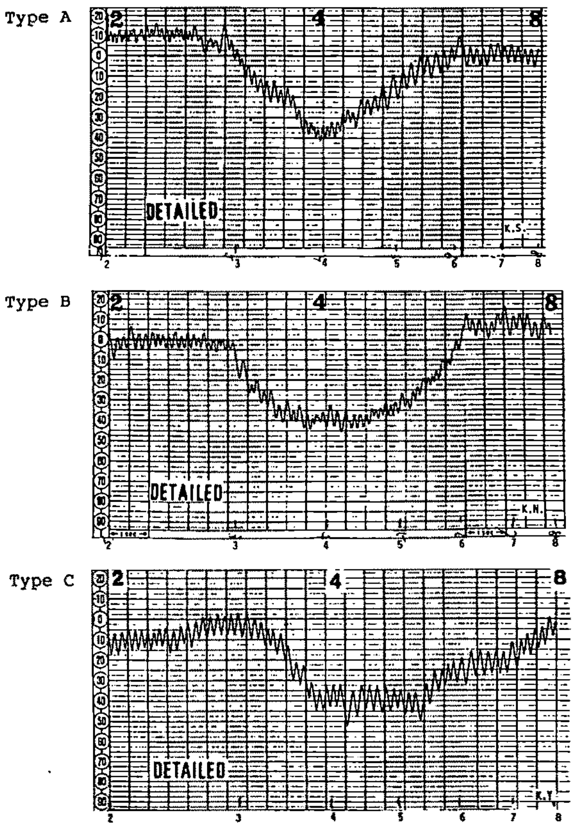

Fig 2. Detailed audiograms of types A, B, and C.

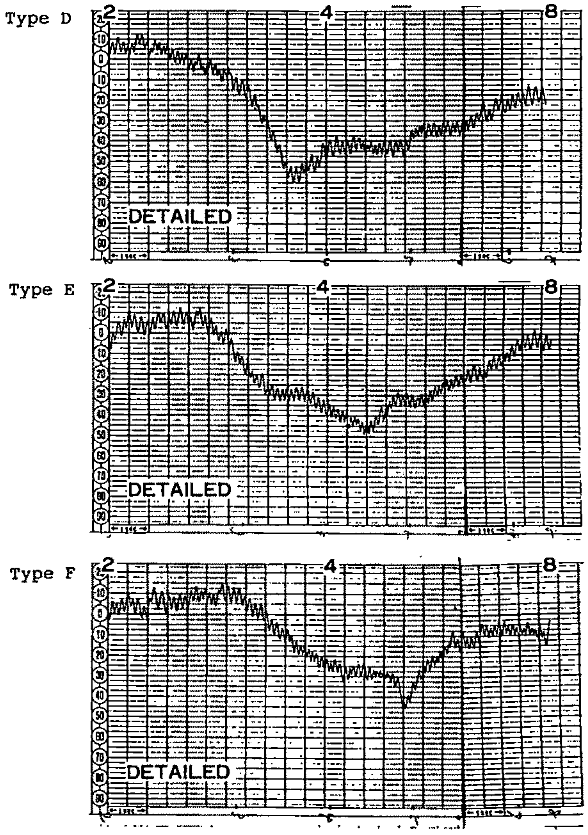

Fig 3. Detailed audiograms of types D, E, and F.

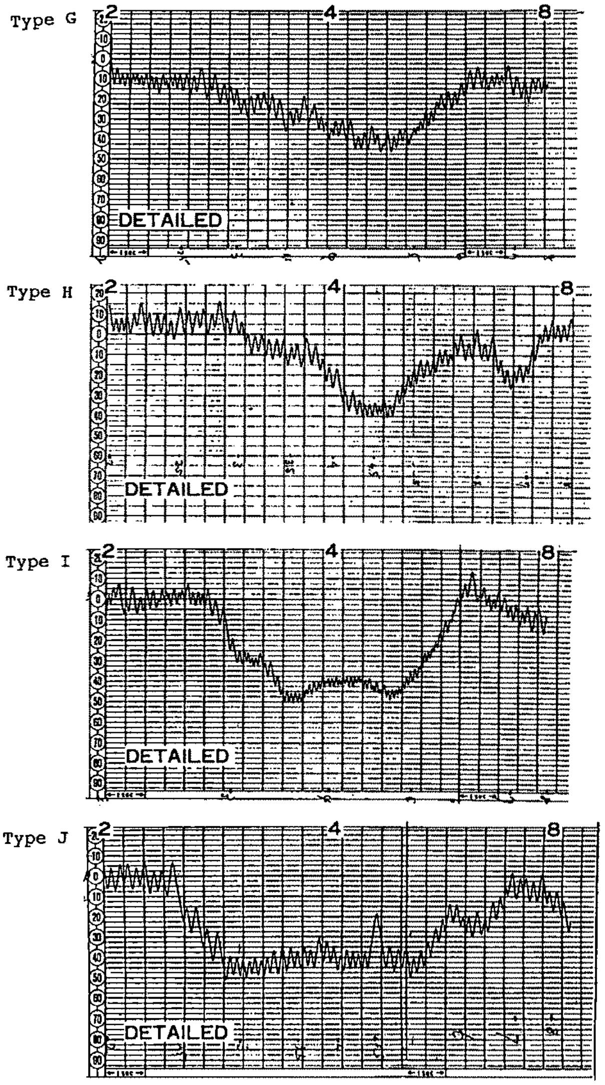

Fig 4. Detailed audiograms of types G, H, I, and J.

H — Cases in which the microdip is seen at another frequency range (high frequency range) outside the dish type (Fig 4).

I — Cases in which the form shows a W shape, by separating a dish type into two parts (Fig 4).

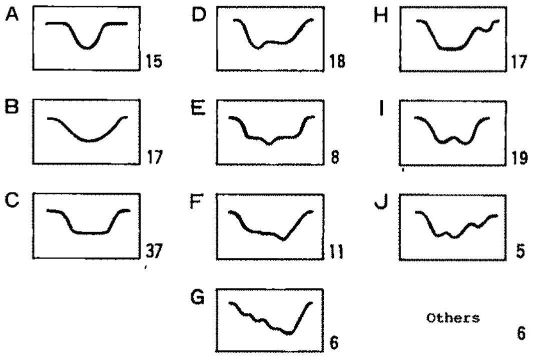

Fig 5. Types of detailed audiogram and number of cases.

TABLE 1. INCIDENCE OF DIP TYPES BY CAUSE OF HEARING LOSS

	Noise-Induced		*Unknown*		*Familial*		
Types	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Total*
A	6	7.9	8	12.9	1	4.8	15
B	7	9.2	6	9.7	4	19.0	17
C	17	22.4	17	27.4	3	14.3	37
D	9	11.8	7	11.3	2	9.5	18
E	6	7.9	2	3.2	0	0	8
F	5	6.6	5	8.1	1	4.8	11
G	3	3.9	2	3.2	1	4.8	6
H	7	9.2	5	8.1	5	23.8	17
I	10	13.2	6	9.7	3	14.3	19
J	4	5.3	1	1.6	0	0	5
K	2	2.6	3	4.8	1	4.8	6
Total	76		62		21		159

J — Cases in which the microdip is seen at another frequency range (high frequency range) outside the W shape (Fig 4).

K — Other forms.

While the configurations of the dips on the detailed audiograms could be classified into the above 11 groups, they were all based on 3 specific types — A, B, and C (Fig 2). In the other dip types, a narrow, wedgelike deterioration area of hearing threshold (microdip) was superimposed on some portions of these 3 basic types, and the smooth, curved portion or the flat horizontal portion of these 3 basic types disappeared, or a microdip was observed at another frequency range of these curves.

Incidence of Each Type on Detailed Audiograms. These 11 groups and their frequency of occurrence are shown in Fig 5. The most common type, C, was recorded in 37 patients, followed in order of decreasing frequency by types I, D, B, and H. However, a total of 69 patients (43.3%) showed smooth curves (A, B, and C) on the detailed audiogram without any microdip, while the detailed audiograms of all the other groups were types modified by microdips, and did not always show a smooth curve. The mean ages

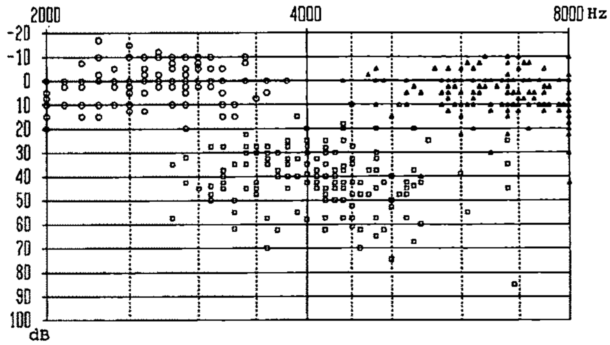

Fig 6. Distribution of frequencies at beginning, bottom, and end of dip in hearing threshold.

TABLE 2. MEAN FREQUENCY IN HERTZ AT BEGINNING, BOTTOM, AND END OF DIP

	Beginning	*Bottom*	*End*
Noise-induced	2,740	4,139	6,833
Unknown	2,753	4,238	6,735
Familial	2,581	4,448	6,590
Means	2,724	4,218	6,762

of the 2 groups, those with types A, B, and C, and those with the other types, from D to K, were 35.3 years and 38.0 years, respectively; while the mean age of the latter was slightly higher, there was no clear difference between the groups.

Distribution of Detailed Audiograms by Cause. The frequency of occurrence of the various patterns, in the 159 patients, is tabulated according to cause of hearing loss in Table 1.

Types C and I were somewhat more frequent in the cases with noise exposure, types C and A in the cases whose cause was unknown, and types H and B in the cases of familial hearing loss, and no clear characteristic findings were detected in any of the groups, because they all seemed to exhibit essentially the same tendencies.

Frequencies at Beginning, Bottom, and End of 4,000-Hz Dip. I recorded the frequencies at which the curves on the detailed audiograms started downward (frequencies at the beginning of the dip), the frequencies at which the thresholds reached the bottom (highest hearing thresholds), and the frequencies at which the curves reached the upward end (frequencies at the end of the dip) on the detailed audiogram in Fig 6. The circle represents the frequency at the beginning of the dip; the triangle, the frequency at the end of the dip; and the square, the maximum hearing threshold. In the dish-type cases, the maximum hearing threshold is the threshold at the center of the horizontal part. Table 2 shows the mean frequencies at these 3 points according to the categories of patients. The mean frequencies at the 3 points, in all patients, were 2,724 Hz, 4,218 Hz, and 6,762 Hz. Among the mean frequencies at these 3 points, the frequency at the beginning of the dip was in a slightly lower frequency range in the patients with familial hearing loss than in the other 2 groups. The maximum hearing threshold was in a higher frequency range, and the frequency at the end of the dip was in a lower range; however, there were no clear statistically significant differences on the correlation coefficient test.

TABLE 3. MEAN SLOPE IN DECIBELS PER OCTAVE OF DOWNWARD AND UPWARD DIPS ACCORDING TO CAUSE OF HEARING LOSS

	Downward Dip	*Upward Dip*
Noise-induced	117.3	79.3
Unknown	105.6	79.3
Familial	70.8	76.4
Means	105.7	79.3

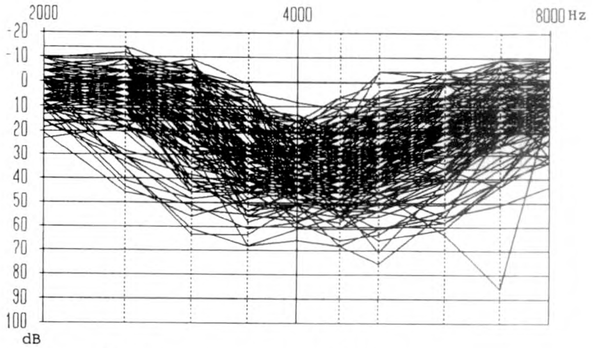

Fig 7. Superimposed detailed audiograms.

Slope of Descending and Ascending Curves. I measured the mean slope of descending curves and ascending curves. The results according to patient categories are shown in Table 3. The mean slope of the descending curve (105.7 dB per octave) was higher than that of the ascending curve (79.3 dB per octave). The descending slope of the patients with familial hearing loss (70.8 dB per octave) was lower than that of the other 2 groups, but no clear differences were detected in the ascending slopes.

Mean Hearing Level. Hearing thresholds from the detailed audiograms at 10 frequencies — 2,000, 2,500, 3,000, 3,500, 4,000, 4,500, 5,000, 6,000, 7,000, and 8,000 Hz — are superimposed in Fig 7. The pattern is almost perfectly symmetric around 4,000 Hz, but there was 1 patient in whom the maximum threshold was at 7,000 Hz. Figure 8 shows the mean audiograms. The hearing levels of the patients with familial hearing loss were slightly lower than, but almost the same as, those in the other 2 groups.

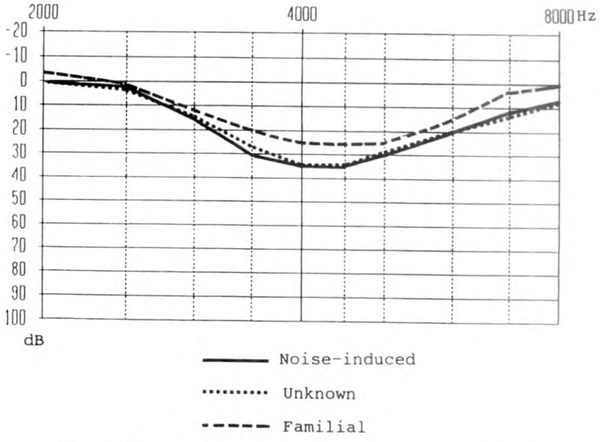

Fig 8. Mean audiograms by cause of hearing loss.

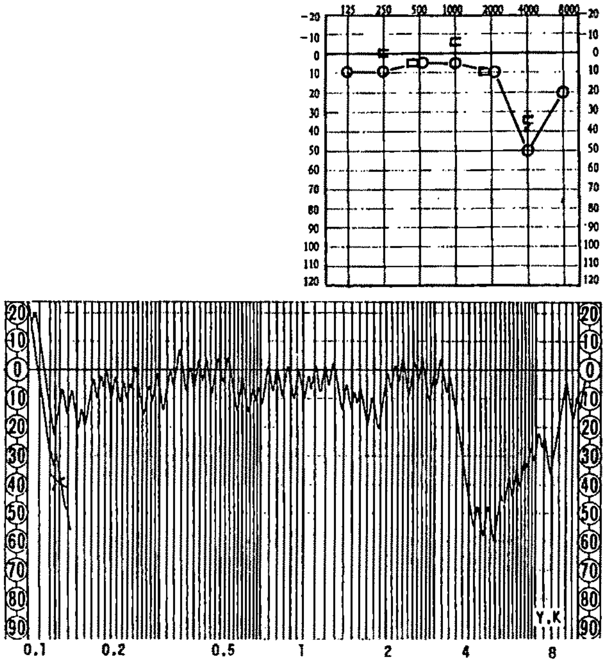

Fig 9. Pure tone audiogram and self-recording audiogram.

DISCUSSION

There are few reports concerning detailed studies on the properties of the dips in pure tone audiograms.

The 4,000-Hz dip seen on pure tone audiograms is well known as the type observed in the early stages of noise-induced hearing loss or in acoustic trauma, but these dips are not always caused by noise exposure. They are known to occur in various diseases, including viral infections, head injuries, hereditary hearing loss, ototoxicity, acoustic trauma, acoustic neuroma, perilymphatic fistulas, and barotrauma, and in many cases the cause is unknown.

A number of papers have been published on pathologic findings in the inner ear when the 4,000-Hz dip is observed. In a study on temporal bone lesions in 3 patients exhibiting a dip at 4,000 Hz induced by acoustic trauma, Igarashi et al[2] reported that the greatest damage was seen in the organ of Corti in the portion 10 to 12 mm from the end of the basal turn. Bredberg[3] similarly reported observing a loss of the basal turn of the organ of Corti and the radial fibers. The 4,000-Hz dip has, therefore, been thought to represent damage with a predominant lesion occurring in the hair cells of the inner ear. However, since the dips seen on the audiograms display similar patterns, regardless of which above-mentioned diseases are present, it is difficult to judge the clinical differences on the basis of the pure tone audiogram alone.

Several methods have been used to examine the 4,000-Hz dip in detail, principally Bekesy's self-recording audiometry and the detailed audiometry of Dishoeck and Gool.[4] Bekesy's self-recording audiometry is widely used in routine clinical practice. In this method, the thresholds are continually measured as the frequencies are changed continuously along the intensity scale. In detailed audiometry,[4] the thresholds are measured as the test tone is changed along the frequency scale at a fixed tone level. However, this results in poor efficiency of measurement, and errors in the measurement methods have not yet been investigated. Therefore, the method of Dishoeck and Gool has not been routinely used. On the other hand, with Bekesy's self-recording audiometry, when the frequency characteristic curve of a hearing loss exceeds a certain slope due to the measurement conditions, accurate measurement of the thresholds in that area becomes impossible. The limit of measurement in such cases is said to be 180 dB per octave. Figure 9 is an audiogram measured by routine self-recording audiometry in a patient with a 4,000-Hz dip. The curve of the hearing threshold shows a straight line in the portion where the thresholds start downward at 3,000 Hz; this means that because the threshold changes in relation to frequency in the portion where the thresholds are rising too steeply, the correct thresholds are not being measured in this frequency range. Thus, the frequency changes must be slowed to enable accurate measurement of the threshold.

In 1974, Tsuiki et al[1] devised detailed audiometry as a means of recording threshold changes. This method differs from routine self-recorded audiometry in that the rate of change in frequency is set at about one third (5 minutes and 40 seconds per octave) of the ordinary rate, and the rate of paper speed is set at 8.5 cm per octave. As a result, the intensity limit of measurement is 640 dB per octave, making it possible to correctly measure almost all cases of the threshold changes in dip-type audiograms. Tsuiki et al described the audiograms obtained with this method as being "detailed."

Dip-type audiograms have a deep indentation indicating a sharp hearing loss at 4,000 Hz on conventional pure tone audiograms. However, on detailed audiograms with the frequency scale drawn out, this configuration was recorded in several patterns. These configurations were roughly classified into 2 groups: one with smooth curves (3 types — A, B, and C, referred to as group 1 below) and a group of types in which a microdip (Tsuiki et al[1] referred to small, sharp depressions observed on continuous recording of Bekesy's audiogram as microdips) is superimposed on this curve and the curve is indented (types D to K, referred to as group 2 below). Examination of the frequency of these 2 groups showed that the former appeared in 69 (43.4%) and the latter in 90 (56.6%) of the 159 subjects, with a slightly higher frequency of occurrence in group 2 of audiograms with microdips appearing in the curves. However, the basic types of curves exhibiting notches, ie, curves with microdips superimposed, were A, B, or C, and group 2 appeared to represent either cases in which partial damage was superimposed on these 3 types or cases in which damage occurred in other frequency ranges.

From the examination of detailed audiograms, Tsuiki[5] found that the dip-type audiograms seen in pure tone audiograms represented extremely sharp wedge-shaped or trapezoidal loss of sensory cells occurring on the basilar membrane, that the pattern of the pure tone audiogram was determined by the circumstances of its distribution, and that the damage was due to the segmental degeneration of cochlea sensory cells. The blood vessels of the organ of Corti are known to form loops, but the loops have a segmental structure, and damage to these vessels is thought to cause segmental degeneration of the sensory cells. Moreover, the configuration of the resulting dip can be expressed in variable forms.

As stated above, dip-type audiograms are seen in a variety of diseases, and the forms of the dips seen on pure tone audiograms are usually of the same type. In the present study, I classified the 159 patients into 3 groups according to cause, but failed to detect any characteristic configurations of dip in these 3 groups. I suspect that as a result, even though there were differences in the pathologic conditions causing the segmental degeneration, the patterns of the dips on the audiograms were of a similar type.

I also investigated the frequencies at the beginning and the end of the dip on detailed audiograms, but found no major differences between the mean frequencies of the 3 subject groups. However, there were significant individual differences in the frequencies at the beginning and end of the dip, with frequencies at the beginning of the dip being widely distributed between 2,000 and 4,000 Hz. A similar tendency was observed in the frequencies at the end of the dip, with a distribution ranging from close to 4,000 Hz to below 8,000 Hz. On the other hand, the minimum and maximum thresholds of the dips were also widely varied, extending from about 2,700 Hz to close to 8,000 Hz. There were also cases in which a microdip was observed at a different frequency range, as in type H and type J, but the diversity of these dips was understood to represent the breadth of segmental degeneration as described above. When I measured the thresholds at the 10 frequencies from 2,000 to 8,000 Hz, the curve of hearing thresholds showed a symmetric pattern centered at around 4,000 Hz in almost all cases. This would seem to indicate that the greatest segmental degeneration is centered on this frequency.

Several hypotheses concerning the pathogenetic mechanism of hearing loss characterized by 4,000-Hz dips can be cited, including those stating that the theory is due to external auditory canal resonance, or the physical properties of waves arising in the cochlea, or the distinctive characteristics of the metabolic structure of the cochlea, even though many details regarding this remain unclear. On the other hand, it is also stated that many dips seen in pure tone audiograms are not centered at 4,000 Hz, but are observed at 5,000 and 6,000 Hz.

In this study, I have described the hearing loss characterized by dips seen at 4,000 Hz on detailed audiograms, and assessed the damage. In the future, it is hoped that hearing loss will be explained in terms of clinical audiology by identification and analysis of the lesions in the inner ear by these methods.

CONCLUSIONS

I conducted a detailed investigation of the 4,000-Hz dip seen on a pure tone audiogram, using the detailed audiometry of Tsuiki et al,[1] and obtained the following results.

1. The dip configurations observed in the detailed audiograms were classified into 11 different types, the cone type, bowl type, and dish type being the 3 basic types.

2. The other types consisted of small dips (microdips) superimposed singly or multiply on these basic configurations, which are thought to be formed by further modification of microdips as the result of a variety of combinations.

3. No clear differences in dip characteristics according to the diseases thought to be causing the dip were observed, and the dips appeared to display similar configurations regardless of the cause of hearing loss.

ACKNOWLEDGMENT — I am very much indebted to Professor Takashi Tsuiki for his useful advice.

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AUDITORY EPITHELIAL MIGRATION

III: AN IMMUNOHISTOLOGIC STUDY USING ANTI-BRDU ANTIBODY ON TYMPANIC MEMBRANE IN MOUSE

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A localization pattern of epidermal cells on the tympanic membrane (TM) and their migratory patterns were studied in mice, by means of immunohistologic technique using an anti-bromodeoxyuridine (BrdU) antibody. The BrdU was instilled intraperitoneally and the animals were painlessly sacrificed between 1 hour and 10 days after the injection. An immunostaining technique using anti-BrdU antibodies was applied on whole mount TM tissues. One hour after injection, BrdU-labeled cells were found in the handle of the malleus (HM) region and in the annular region of the pars tensa of the TM. Some labeled cells were observed in the intermediate region of the upper half of the superior quadrant, but no labeled cells were found in the remaining part of the intermediate region. Labeled cells were also evident in the pars flaccida without any particular pattern of distribution. As time elapsed after the injection, the labeled cells first appearing in the HM region had migrated laterally and inferiorly from the HM toward the annulus, while those in the annular region had considerably decreased in number. Results of the present study are the following: 1) the proliferation center of epidermal cells in the pars tensa is located in two different areas, ie, the HM region and annular region, 2) newly generated cells in the HM region migrate from the HM region toward the annular region, whereas those in the annular region migrate from the annular region to the external auditory canal, and 3) no specific generation center is located in the pars flaccida. On the basis of these results, we discuss the relationship between the site of the proliferation center of epidermal cells and their migratory patterns.

KEY WORDS — BrdU immunohistochemistry, epidermal cell, epithelial migration, generation center, mouse, tympanic membrane.

INTRODUCTION

The physiological functions of epidermal cell migration on the tympanic membrane (TM) and external auditory canal (EAC) are considered to be at least two: cleansing, and healing. Under normal conditions, epidermal cells migrate from the center of the TM toward the annulus to keep the surface of the TM free from debris, cerumen, and foreign materials. In a pathologic condition such as TM perforation, epidermal cells also migrate to repair the TM.

The migratory pattern of the auditory epidermal cells in the guinea pig[1-3] has been reported to be quite different from that in the human.[4-7] We have therefore reported on patterns of the auditory epithelial migration (AEM) in the rat visualized by means of a dye marking technique.[8] This species shows almost the same pathway patterns as found in the human.[4-7] Due to this fact, we consider the rat one of the most useful experimental animals for studies of the human type of AEM.

The macroscopic and microscopic features of the TM and EAC in the mouse[9,10] are quite similar to those in the rat.[11] Furthermore, our preliminary study showed movement of dyes on the mouse TM to follow the same pattern as in the rat. The epidermis of both the pars tensa (PT) and pars flaccida (PF) of the TM in the mouse is only two or three cell layers thick; therefore, it may be easier for antibodies to penetrate into tissue. For the above-mentioned reasons, we chose the mouse as an experimental animal for studies of the human type of AEM.

The thymidine analog bromodeoxyuridine (BrdU) is incorporated into DNA during the S-phase of the cell cycle,[12,13] and single-stranded BrdU-labeled DNA can be detected immunohistochemically.[14,15]

The purpose of this study was to locate the site of the proliferation center of epidermal cells on both the PT and PF of the TM by applying a BrdU immunostaining technique, and to identify the migratory pattern of epidermal cells. We also discuss the relationship between the pattern of labeled cell migration and the pattern of dye movement.

MATERIALS AND METHODS

Experiment 1 (BrdU Immunostaining on Whole Mount TMs). A total of 60 ears from 30 mice were

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used in this study. We dissolved BrdU (Serva, Heidelberg, Germany) in physiological saline (0.9% sodium chloride) to obtain a final concentration of 10 mg/mL. Twelve groups of 2 mice each were injected intraperitoneally with BrdU (50 mg/kg body weight) at 1, 2, 3, 6, 12, and 24 hours and 2, 3, 4, 5, 7, and 10 days before sacrifice. All injections were carried out between 2 and 4 PM to exclude any effect of diurnal rhythm on the mitotic activity of the epidermal cells.[16] Three non–BrdU-injected animals and another 3 injected with BrdU 1 hour before sacrifice were used as negative controls.

After decapitation, the temporal bones were immediately removed. Each mastoid bulla was opened inferiorly and ice-cold 80% ethanol was instilled into the middle ear cavity and the EAC with a 1-mL syringe. The specimens were immersed overnight in 80% ethanol at 4°C. After the 80% ethanol was washed out with 0.01 mol/L phosphate-buffered saline (PBS), the specimens were treated with 0.4% Triton-X (United States Biochemical Co, Cleveland, Ohio) in 0.01 mol/L PBS for 15 minutes to improve antibody penetration. To convert double-stranded DNA into single-stranded nucleic acid, the specimens were treated with 2N hydrochloric acid at room temperature for 1 hour, and then neutralized in 0.01 mol/L PBS for 30 minutes. At this stage, the TMs were carefully removed from the temporal bones. After rinsing with 0.01 mol/L PBS, the immunostaining protocol described elsewhere[17] was applied to the whole mount tissues of the TMs. Each tissue specimen was placed individually into a 1.2-mL plastic tube and each reagent solution was gently poured into the tubes through a micropipet. Anti-BrdU sheep polyclonal antibodies (Fitzgerald, Concord, Mass) diluted 1:40 in 0.01 mol/L PBS containing 3.0% bovine serum albumin (BSA) and 0.4% Triton-X were allowed to react with TMs at 4°C for 20 hours. The antigen-antibody mixture was also used on TMs of both non–BrdU-injected animals and animals injected with BrdU 1 hour before sacrifice as a preadsorption control. After rinsing with 0.01 mol/L PBS containing 0.4% Triton-X for 1 hour and then 0.01 mol/L PBS for 1 hour, threefold biotinylated donkey anti sheep IgG antibody (Sigma, St Louis, Mo) diluted 1:100 in 0.01 mol/L PBS containing 3% BSA and 0.4% Triton-X was applied to the specimens at 4°C for 20 hours. After rinsing with 0.01 mol/L PBS containing 0.4% Triton-X for 1 hour and then 0.01 mol/L PBS for 1 hour, three times, 0.3% hydrogen peroxide in methanol was applied for 30 minutes to block any endogenous peroxidase reaction. After rinsing with 0.01 mol/L PBS for 1 hour, an avidin-biotin-peroxidase complex (ABC; Dako, Grastorp, Denmark) solution containing 0.4% Triton-X was allowed to react with the TMs at 4°C for 20 hours. The tissues were finally rinsed with 0.01 mol/L PBS containing 0.4% Triton-X twice for 1 hour, and then twice with 0.01 mol/L PBS for 1 hour and were then exposed to 3,3'-diaminobenzidine-tetrahydrochloride (DAB; Sigma) for 30 minutes. To stop the DAB reaction, the DAB solution was replaced by PBS at 0.01 mol/L. The tissues were mounted in Mowiol (Aldrich-Chemie, Steinheim, Germany). Light microscopic observation was performed with a Zeiss Axiophot microscope (Germany). The labeled cells could be observed at ×50 magnification under a stereoscope and sketched on the diagrams.

Experiment 2 (BrdU Immunostaining on Colonic Sections). As a control to check whether or not BrdU was taken up effectively into the nuclei of dividing cells, the ascending colon was collected from each animal. The samples were rinsed with physiological saline to wash out stools and immediately immersed in ice-cold 80% ethanol at 4°C and left overnight. Subsequent treatment with absolute ethanol for 30 minutes and xylene for 20 minutes was performed twice. The tissues were then embedded in paraffin wax, sectioned 4 μm thick, deparaffinized with xylene, and rehydrated through a descending series of ethanols. After rinsing with 0.01 mol/L PBS, the sections were treated by the ordinary immunostaining method using anti-BrdU antibody as described elsewhere.[17] Each antibody was diluted to the same concentration as used in experiment 1. The sections were incubated in each antibody for 20 minutes and then in ABC solution for 20 minutes and finally reacted with DAB solution for 5 minutes. These procedures were carried out at room temperature. The immunostained sections were mounted in Eukitt (Kindler, GmbH & Co, Freiburg, Germany) without counterstaining of nuclei.

RESULTS

Experiment 2 (Control Tissue; Ascending Colon). The absorbed antisera serving as the preadsorption control showed no immunoreactivity in either the non–BrdU-injected colonic sections or the BrdU-injected ones (data not shown). In all of the colonic tissues from BrdU-injected animals, numerous BrdU-labeled cells were found in the glandular mucosa. At 1 hour after injection (Fig 1A), the labeled cells were located mainly at the base and lower part of the intermediate zone of the glands. They were then moving toward the luminal surface as time elapsed, and some labeled cells were found at the luminal surface 2 days after injection. At 4 days (Fig 1B), labeled cells were found at the luminal surface and the intermediate zone, though they were also found at

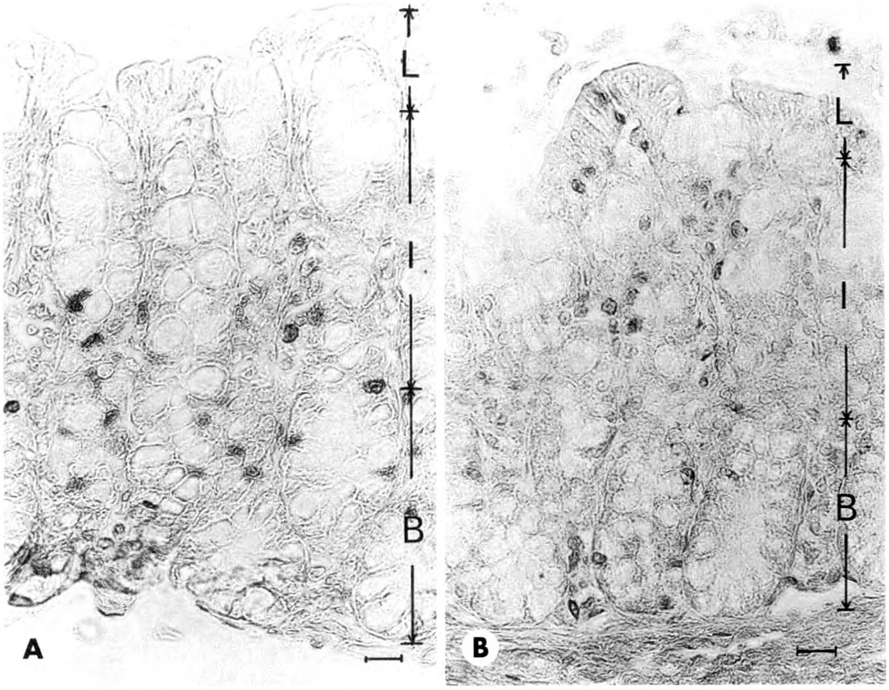

Fig 1. Bromodeoxyuridine (BrdU)–labeled cells in ascending colon. Bars — 20 µm, B — basal part of gland, I — intermediate part, L — luminal surface (apical part). A) One hour after injection. B) Four days after injection.

the base of the gland. At 5 to 6 days after injection, no labeled cells could be found in the colonic tissues.

Experiment 1 (Tympanic Membrane). The BrdU was effectively displayed in nuclei of the mitotic cells on the TMs of all injected animals. To find out which layer of the cells it was that displayed BrdU, careful examination under phase contrast conditions of an Axiophot microscope was carried out, and most BrdU-labeled cells were found in the epidermis of the TM. No labeled cells were seen in the negative control tissues. The pattern of positive immunoreactivity was of two types (Fig 2): 1) the homogeneous type, in which the nucleus is entirely and uniformly stained brown and contains numerous tiny dark brown dots, and 2) the granular type, in which the nucleus is unstained except for the membrane and contains several coarse black granules. The number of BrdU-labeled cells in each TM varied from approximately 100 to 250 in the PT and from approximately 30 to 80 in the PF.

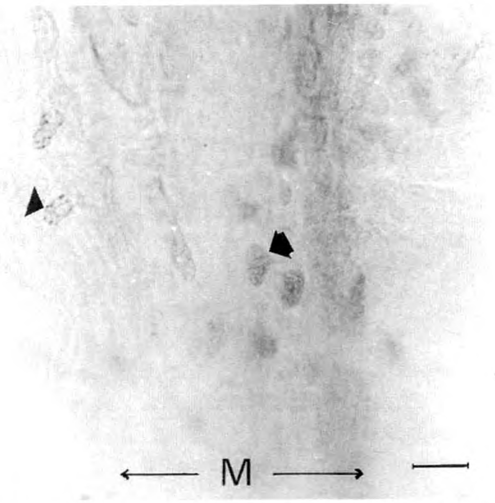

Fig 2. BrdU-labeled cells on surface of handle of malleus (M) in left tympanic membrane 1 hour after injection. Two types of labeled cells are displayed: homogeneous type (arrow) and granular type (arrowhead). Bar — 10 µm.

One to 6 hours after injection (Fig 3), in most TMs, labeled cells in the PT were found near the handle of the malleus (HM; Fig 3C), especially at its upper half, and also near the annulus (Fig 3D). In only 1 of 16 TMs removed 1 to 6 hours after injection were most labeled cells located in the umbo region instead of the upper half of the HM region (Fig 4). In the upper half of the superior quadrant of the PT, some labeled cells were additionally found in the intermediate region between the HM and the annulus (Fig 3C), but no labeled cells were found in the remaining part of the intermediate region. In the PF, the labeled cells were found without any specific distribution pattern (Fig 3B).

On day 3, the labeled cells had already started to migrate from the upper part of the HM region (the generation center) toward the annulus (Fig 5). By 9 days after injection, most labeled cells were found in the middle and lower parts of the PT (Fig 6). As time went on, the cells were migrating even further from the generation center. The labeled cells visible in the annular region were remarkably decreased in number by day 5 and showed no tendency to migrate toward the center of the PT. In the PF, the labeled cells were still found at day 10, and their random distribution

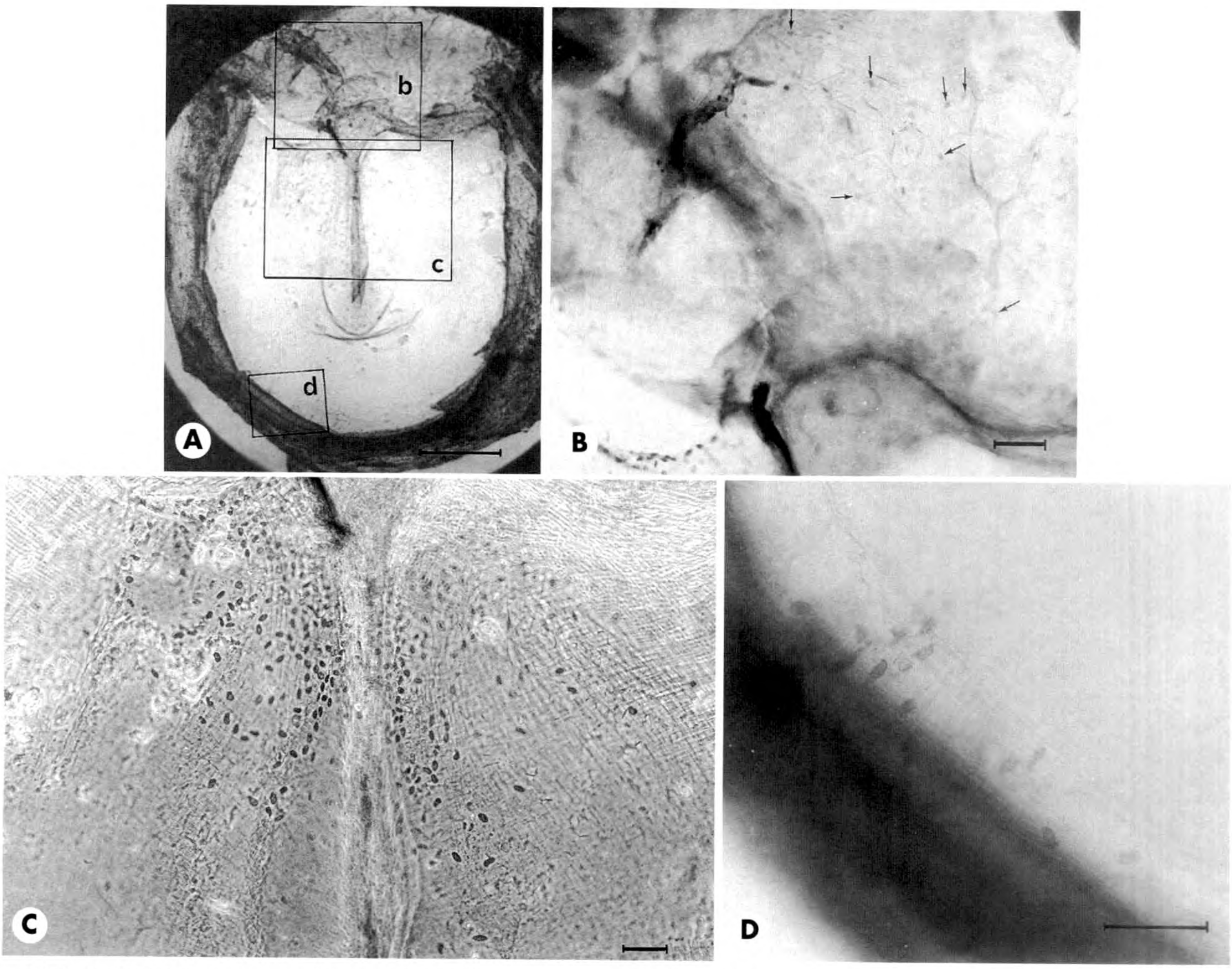

Fig 3. BrdU-labeled cells in right tympanic membrane at 3 hours after injection. A) Whole view. Bar — 0.5 mm. B) Pars flaccida (b on A). Bar — 0.1 mm, arrows — labeled cells. C) Superior quadrant (c on A). Bar — 0.1 mm. D) Annular region (d on A). Bar — 50 μm.

pattern remained the same throughout the study.

We could elucidate the direction of the migratory pathway of labeled cells by analyzing the detailed changes of the distribution patterns. The labeled cells first appearing in the HM region migrated laterally and inferiorly toward the annulus, and those first found in the annular region migrated toward the EAC and finally appeared in the annular region of the EAC. The BrdU-labeled cells in the PF probably migrated upward from the basal layer to the superficial layer.

A summary of the results from the present study regarding the distribution patterns of the BrdU-labeled cells on the TM and the ascending colon are represented in Fig 7, and the migratory pattern of labeled cells is summarized in Fig 8A.

DISCUSSION

How can a dye dot move over the TM and EAC? Most otologists know of this phenomenon, but know little about its mechanism. In order to solve this problem, we must at first elucidate whether or not there is a relationship between the movement of dye markings on the TM and the migration of BrdU-labeled cells in the epidermis of the TM. The reason for this is that the results from the dye marking studies[1,4-7,18] represent only the movement of the outermost layer of the TM, but those from the labeled cell studies[1,2,19] reveal the migration pattern of epidermal cells in the deeper layer of the epidermis of the TM. Furthermore, as all migration studies in the human[4-7] were performed by observing the movement pattern of dye markings, we have to establish the above-mentioned relationship in order to estimate the migratory pattern of epidermal cells from the results of dye marking studies. Such attempts have earlier been performed in guinea pigs[1,2] and in the mouse.[19] Fortunately, in the guinea pig, the results of a macroscopic study[1] are the same as those of autoradiographic studies.[1,2] However, in the mouse, an earlier autoradiographic study[19] indicated a site for

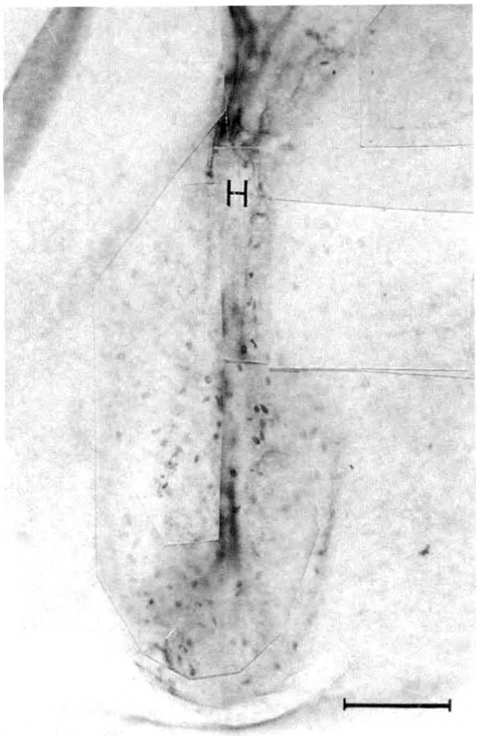

Fig 4. Umbo type of generation center in right tympanic membrane at 2 hours after injection. Bar — 0.2 mm, H — handle of malleus.

the generation center of epidermal cells on the TM, whereas this study showed a slight tendency of the labeled cells to move slowly from the annular region toward the center of the PT. We have already investigated the pattern of the movement of dye markings on the PT and PF of the TM in the rat,[6] and we also have some preliminary data on the PT of the mouse. From these studies, there are reasons to believe that the movement pattern of markings is the same in the rat and the mouse (Fig 8A); ie, most dye spots move laterally and inferiorly from the HM toward the annulus. In our present study, we could show the results photographically and establish that one generation center is situated near the HM and another in the annular region of the PT. It is also evident that newly generated cells in the HM region migrate laterally and inferiorly from the generation center to the annulus. In the mouse, the dye movement can be used as a substitute for the migration of epidermal cells in the deep layer of the epidermis of the TM. Probably, we can draw the same conclusion in the human as in the guinea pig and the mouse. Most probably, we can consider Boedts' data[19] to be correct, though the high sensitivity of detection of the labeling by autoradiog-

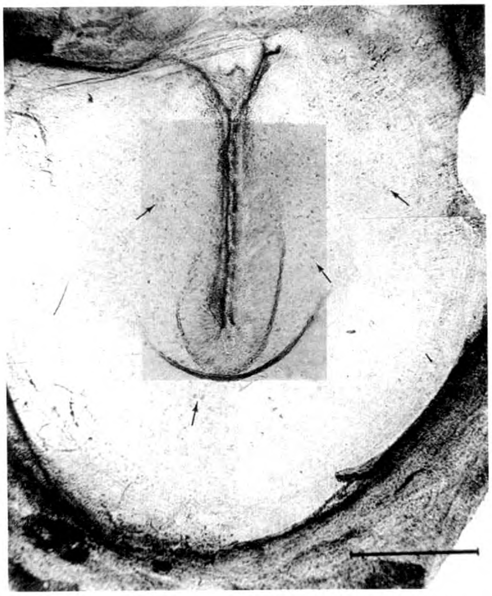

Fig 5. BrdU-labeled cells in right tympanic membrane at 3 days after injection. Labeled cells (arrows) are migrating inferiorly and laterally from handle of malleus region. Bar — 0.5 mm.

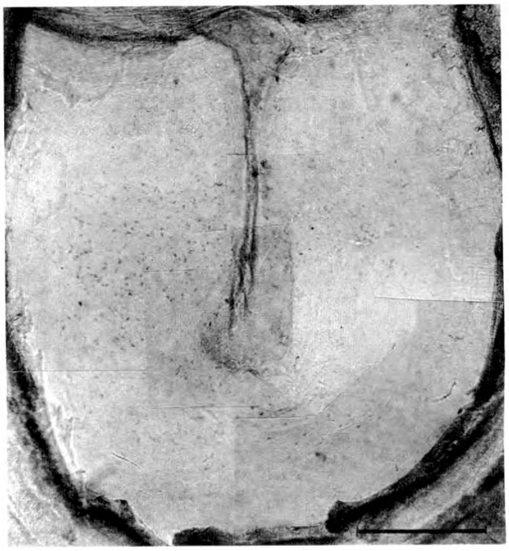

Fig 6. BrdU-labeled cells in left tympanic membrane at 9 days after injection. Only few labeled cells were found in upper half of superior quadrant. Instead, most labeled cells were situated in lower half of superior quadrant and inferior quadrant. Bar — 0.5 mm.

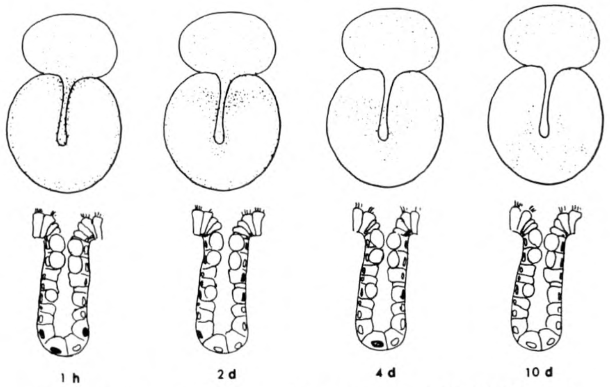

Fig 7. Scheme of expression patterns of BrdU-labeled cells on tympanic membrane (top row) and ascending colon (bottom row). Time since injection is shown.

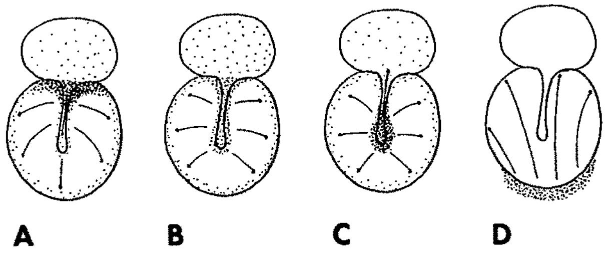

Fig 8. Scheme of different patterns of generation center location (dots) and migratory pathways (arrows). A) Upper part of handle of malleus type. B) Mixed type. C) Umbo type. D) Sinus meatus type.

raphy could cause a problem in analyzing the findings. If we could ignore the early-appearing labeled cells in the annular region, we would no doubt identify the probable migratory pathways from Boedts' results.

The amount of BrdU incorporated into the DNAs (nucleic acids) of the first-generation cells (those in S-phase at the time of BrdU injection) may be halved with each subsequent division. The possibility of detecting BrdU-labeled cells after repeated cell divisions therefore depends mainly on the sensitivity of the method of incorporating BrdU into the nuclei. Our immunostaining method used on colonic tissues could not detect labeled cells in paraffin-embedded colonic sections after two or three rounds of cell division. It would appear that antibodies are less capable of penetrating into whole mount TM tissues than into sectioned tissues. Hence, we are likely to detect BrdU only in first- or second-generation cells in whole mount TMs. The area where labeled cells first emerge is considered the generation center, and the site where cells first disappear is also the generation center.

In the guinea pig, the generation center of epidermal cells of the TM is located at the inferior wall of the deep EAC,[1,2] and newly generated cells migrate upward via the TM to the lateral and upper walls of the EAC (called "the sinus meatus type" by our classification; Fig 8D).[1-3] In contrast, the mouse has been reported to display uniform epidermal cell proliferation in the TM.[20] According to our results, in the mouse, there are two different well-circumscribed generation centers in the PT: 1) the center of the HM region and 2) the center of the annular region. In the human, mitotic figures of the epidermal cell have been reported in the umbo region,[21] and expression of cytokeratin 19 in the basal epidermal cells in the annular region of the PT[22] may also indicate the proliferative capacity of the annular region. We have established a justification of the above-mentioned supposition by an immunohistochemical approach using anti–proliferating cell nuclear antigens, Ki-67 and PCNA.[23] The location of the generation center of epidermal cells in the PT is the same in the mouse and human,[23] and the migratory patterns of epidermal cells in the mouse and human[4-7] are also almost identical. The difference in the basic pattern of AEM between the human, mouse, and guinea pig may grossly depend on the difference in the site of the generation center. In the superior quadrant, first-generation cells are also found in the intermediate region between the HM and the annulus. Our preliminary embryological study has shown that the origins of all parts of the PT are identical. We therefore believe that this intermediate region is an extension of the generation center of the annular region.

However, minor differences in the migratory pattern along the HM have been reported even in different individuals of the human.[5,6] The patterns of AEM have been studied in 68 young male students by observing dye movement.[5] Two broad patterns of migration were observed: 1) type 1 occurred in about 80%, showing pathways radiating from the umbo toward the periphery; 2) type 2 occurred in less than 20%, having a similar pathway to type 1 in the inferior quadrant but a different pathway at right angles to the long axis of the HM to the periphery in the superior quadrant. Both types displayed an upward pathway along the HM. We have already described the migratory pathways in the rat[8]; one is similar to type 2 in the human, apart from a downward pathway along the HM instead of the upward one as described in the human. In this study and our preliminary dye marking study, the migratory patterns of epidermal cells in the mouse and rat are mainly the same.

In the mouse, the direction of the migration along the HM is generally downward. We have studied the morphologic structures of the TM and EAC in both the mouse[9,10] and rat,[11] and reported the appearance of "fingerlike projections" as possible morphologic evidence of the migration of epidermal cells in the TM.[9-11] These "fingerlike projections" can provide information about the direction of the migration of epidermal cells in the PT. In rare cases in mice, an upward pathway along the HM could preliminarily be observed, instead of the downward pathway found in most mice. In the mouse, the same condition as occurs in the human is evident regarding the migratory pathway along the HM.

In order to explain two different types of pathways along the HM, an attempt has been made to explain the upward pathway along the HM in the human.[7] It is thought that the origins of the HM region of the PT and the PF are the same, but that the remaining part of the PT has another origin. Hence, the migratory pathway along the HM differs from that of the remaining part of the PT (radially and centrifugally from the HM). According to our embryological studies in the mouse and rat, we believe the origin of the entire PT is identical. Our results showed clearly the HM region was the hub of the generating epidermal cells in the PT of the TM. Additionally, we cannot explain the two different pathways along the HM in mice by the above-described different origin hypothesis. Of course, to explain correctly the causes of these differences in the migratory pathways along the HM, we have to investigate both the movement pattern of dye markings on the TM and the detailed distribution pattern of labeled germinal cells in the HM region in each individual animal. However, according to the present results, we can manage to account for these minor differences in the migratory pattern along the HM by the different subtypes of the generation center in this region. We demonstrate that the generation center in the HM region is furthermore divided into at least two types: 1) the upper part type (Fig 8A), in which most germinal cells are located in the upper half of the HM region, and 2) the umbo type (Fig 8C), in which most germinal cells are in the umbo region. Probably, we can anticipate the existence of a third type (the mixed type, Fig 8B), in which the germinal cells are evenly distributed along the HM. The annular generation center is also found in all three types.

In most mice and rats, the generation center of the HM region is located in the upper half of the HM (Fig 8A), and the pathway along the HM is downward. In a few mice and rats, however, the generation center is located in the umbo region (Fig 8C), and the pathway along the HM is upward. By contrast, in the human, about 80% have the umbo type of generation center (Fig 8C), and all of the pathways disperse radially and centrifugally from the umbo to the periphery. In about 20% of humans, the mixed type of generation center (Fig 8B) is found, and the pathway in the upper quadrant is outward at right angles from the HM to the periphery. The mitotic activity in the umbo is so little higher than elsewhere that the pathway along the HM is slightly upward from the umbo to the PF. In addition, the arrangement pattern of the outermost fibers may relate to the direction of migration in the guinea pig.[24] We agree with this idea, but do not yet have enough data to discuss it.

Auditory epithelial migration also exists in the PF; however, a specific localization of germinal cells could not be found in this study. That the distribution patterns of labeled cells did not change during this study suggests that the newly generated cells directly move upward from the basal to the apical layer and become mature. The mechanism of migration in the PF (radial pathway from the neck of the malleus toward the upper wall of the EAC)[4-7] seems to remain unsolved.

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MAGNETIC RESONANCE IMAGING OF THE DEVELOPMENT OF OTITIS MEDIA WITH EFFUSION CAUSED BY FUNCTIONAL OBSTRUCTION OF THE EUSTACHIAN TUBE

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In this study, magnetic resonance imaging (MRI) was used to define in vivo the effect of experimental functional obstruction of the eustachian tube (ET) on vascular permeability and the development of middle ear (ME) effusion. After collection of baseline data for ME pressure and MRI, the right tensor veli palatini muscle of 10 cynomolgus monkeys was injected with botulinum toxin A to induce ET obstruction. The left tensor veli palatini muscle was injected with saline in 4 monkeys. Right and left ME pressures and compliances were measured twice daily over a follow-up period of 36 days, and MRI scanning sessions including administration of a contrast agent, gadopentetate dimeglumine, were repeated on days 3, 6, 11, 15, 21, 29, and 36 in 6 animals and on days 15, 21, 29, and 36 in 4 animals. Two right ears did not develop underpressures, 5 developed persistent underpressures, and 3 developed underpressures that resolved. No changes in MRI parameters were noted for the ears that did not develop underpressures, but a progressive brightening of the ME on T2-weighted images, indicative of the development of inflammation and effusion, was noted for the others. Also, an increasing rate of transfer of the contrast agent between the vascular and ME compartments, indicative of increasing vascular permeability, was observed to track the temporal changes in ME pressure. These results support a causal relationship between ET dysfunction, ME underpressures, increased vascular permeability, and otitis media with effusion.

KEY WORDS — eustachian tube, magnetic resonance imaging, middle ear effusion, middle ear pressure, otitis media.

INTRODUCTION

Otitis media with effusion (OME) is a commonly diagnosed disease in the pediatric age group and is characterized by inflammation of the middle ear (ME) mucosa and fluid within the ME space.[1] A variety of causes have been proposed for OME, including allergies, bacterial or viral infections, and obstruction of the eustachian tube (ET).[2] With regard to the last, numerous past studies in animals have shown that physical obstruction of the tubal lumen causes ME mucosal metaplasia and an effusion resembling in biochemical composition a transudate from the vascular compartment.[3-7] However, histopathologic studies of autopsy materials do not demonstrate physical obstruction of the tubal lumen in cases of OME.[8-10] This negative evidence was used to discount a role for tubal obstruction in the development of OME. The results of the experimental studies were attributed to either incidental infection of the ME cleft, impairment of the normal mechanism for fluid clearance from the ME, or blockage of the lymphatic drainage of the ME.[10]

The apparent lack of evidence for tubal obstruction in histopathologic specimens with OME is not unexpected. Testing of ET function of children with OME or a history of OME consistently shows free passage of air through the tube at relatively low driving pressures but an inability of these children to open the ET by swallowing.[11-17] Because the ET is usually collapsed by tissue pressures in excess of the ambient environment, these results suggest that a functional failure in the mechanism for tubal dilation is the cause of OME in these children.[11,12] While tubal obstruction is not evaluable in studies of fixed tissues, experimentally created functional tubal obstruction in monkeys has been shown to result in OME. There, impairment of the tubal dilatory mechanism by excision, transection, or paralysis of the tensor veli palatini muscle (TVPm) caused the development of ME underpressures and, if persistent, OME.[18-20] For reversible procedures, such as botulinum paralysis of the TVPm, the OME was observed to resolve following restoration of active muscle function.

The classic mechanism relating ET dysfunction to OME is the hydrops ex vacuo theory, which consists

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of four sequential, causally related events: 1) the unabated absorption of ME gases, 2) the progressive development of ME underpressure, 3) an increased permeability of the mucosa, and 4) a transudation of fluid into the ME space. Recently, the validity of the component assumptions of this theory has been questioned by the results of indirect experiments, and a number of investigators have rejected completely a role for this mechanism as a cause of OME.[21-23]

The monkey model of functional ET obstruction discussed above would seem to be an ideal experimental setting for evaluating the validity of these assumptions under well-controlled conditions. Unfortunately, the high cost of experiments in primates and the previous lack of applicable in vivo tools for assessment of disease progression has limited the usefulness of that model. More recently, studies of OME secondary to bacterial infection using cats and chinchillas have reported that magnetic resonance imaging (MRI) can reveal inflammation and effusion within the ME space, and assess the degree of vascular leakage across the mucosa.[24,25] In the present study, these tools were used for in vivo assessments of the response of the ME to experimentally created functional ET obstruction in cynomolgus monkeys. The temporal sequence of events was evaluated for consistency with the expectations of the hydrops ex vacuo theory.

MATERIAL AND METHODS

Protocol. A total of 10 cynomolgus monkeys (*Macaca fascicularis*) weighing 2.8 to 5.0 kg were studied. In 6 of the animals (cohort 1), the left ear was used previously in other experiments of mucosal blood flow, while the remaining 4 animals (cohort 2) were enrolled immediately after completion of a quarantine period. At entry, the right and left ears were examined by otomicroscopy and tympanometry to document normal ME status. The MEs were also evaluated for the presence of effusion and degree of vascular permeability by means of MRI. Then the right TVPm was injected with botulinum toxin in all animals and the left TVPm was injected with saline in the 4 animals of cohort 2. Tympanometric measures of right ME pressure were made twice daily throughout a 36-day period of follow-up in all animals. The MRI scanning sessions were repeated at 3, 6, 11, 15, 21, 29, and 36 days after induction of TVPm paralysis for cohort 1 animals and at 15, 21, 29, and 36 days for cohort 2 animals. At the completion of the experiments, the animals were donated to other researchers for terminal experiments. The study was approved by the Animal Care and Use Committee at the Children's Hospital of Pittsburgh.

Muscle Injections. The monkey was anesthetized with a mixture of ketamine hydrochloride (50 mg/kg), xylazine hydrochloride (15 mg/kg), and acepromazine maleate (2.5 mg/kg). The animal was placed supine and with the head fixed in position. The mouth was held open by a retractor, and the right hamulus was located by finger palpation. A vertical incision of 1 cm was made from the hamulus posterolaterally. The tendon of the TVPm was visualized and the muscle belly was isolated by blunt dissection. A 0.1-mL solution of botulinum toxin A (Sigma) containing 10 units for cohort 1 and 5 units per kilogram for cohort 2 was injected through a single puncture site along the long axis of the muscle. The mucosal incision was closed with separated silk sutures. In cohort 2 animals, the procedure was repeated for the left side and the left TVPm was injected with 0.1 mL of saline. The animal was observed until recovery and then returned to its cage.

Tympanometry. For tympanometry, the animals were sedated with ketamine hydrochloride (20 mg/kg). Tympanograms were obtained within 10 minutes by means of a Madsen ZO77 clinical instrument coupled to a Grasen Stadler recorder. For each measurement, positive pressures of 200 mm H2O were applied to the external canal and decreased at a rate of approximately 100 mm H2O/s until a defined compliance peak was observed on the tracing. The ME pressure was estimated from the tracing at maximum compliance to an accuracy of 5 mm H2O. The linear response region of the pressure transducer was measured to be from +300 mm H2O to –600 mm H2O. Tympanograms with no obvious compliance peaks were assigned a pressure of –600 mm H2O and a compliance value of 0.

Magnetic Resonance Imaging. For MRI sessions, the animals were anesthetized with a combination of ketamine hydrochloride (50 mg/kg), xylazine hydrochloride (15 mg/kg), and acepromazine maleate (2.5 mg/kg). Middle ear pressures and rectal temperature were measured. A venous line was placed in an extremity vein for later administration of a contrast agent. Animals were transported in an air-conditioned van to the Pittsburgh Nuclear Magnetic Resonance Institute, which is located four blocks from the animal facility. A 1.5-T magnet (Signa, General Electric, Milwaukee, Wis) was used for these studies. The head of the animal was centered in an extremity coil and maintained in position by a custom-made cast. The body was supported to prevent passive movements during the scanning period. A tube containing a solution of known concentration of the contrast agent gadopentetate dimeglumine (0.008 mol/mL) was placed within the coil near the head, the

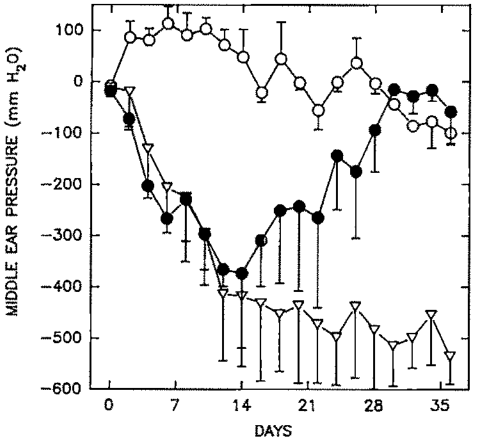

Fig 1. Average middle ear pressures as function of time for subsets of right ears that failed to develop negative pressure (open circles), that developed and resolved negative pressure (closed circles), and that developed sustained negative pressure (open inverted triangles). Vertical bars — 1 SD about mean.

body was covered with a blanket, and the animal was advanced into the bore of the magnet.

For each scanning session, a total of 11 sequences were performed consisting of one sagittal T1-weighted scout scan, one axial T2-weighted scan, one precontrast axial T1-weighted scan, and eight consecutive postcontrast axial T1-weighted sequences. The first T1 sagittal image was used to define the section limits of the temporal bone (TR = 400 milliseconds [ms], TE = 16 ms, thickness = 3.0 mm, space = 1.0 mm, matrix = 256 × 192 mm, field of view [FOV] = 12 cm). For later sequences, the axes of the slices were tilted obliquely to parallel the long axis of the right temporal bone. After the scout scan, a T2-weighted fast spin echo, axial-oblique scan was obtained (TR = 2,200 ms, TE = 104 ms, thickness = 3.0 mm, space = 0.5 mm, matrix = 256 × 256 mm, FOV = 12 cm). Then, the precontrast T1-weighted spin echo scan was performed (TR = 400 ms, TE = 21 ms, thickness = 3.0 mm, space = 0.5 mm, matrix = 256 × 256 mm, FOV = 12 cm). Gadopentetate dimeglumine was administered via the venous line as a bolus at a dose of 0.1 mmol/kg. Postcontrast T1-weighted sequences were begun immediately and repeated eight times without interruption for 30 minutes. Then the animal was placed on a heating pad until it showed signs of recovery, on which it was returned to its cage.

Data Analysis. For each experiment and scan, an image slice containing the maximum area of the pneumatized temporal bone, as well as other visual landmarks such as the cochlea, vestibule, semicircular canals, internal auditory canal, and ET, was selected for study. Inclusion of these landmarks allowed for the definition of identifiable areas for consistent acquisition of intensity information between study sessions. Data were abstracted from the intensity matrices by means of resident software. For every sequence of all scans, the average and standard deviation of the intensity values of all pixels contained in designated regions of interest (ROIs) of approximately 40 mm^2 localized on the index solution, brain tissue, anterior (petrous) portion of the temporal bone, and posterior (mastoid) region without including the extratemporal tissues, labyrinth, ET, or carotid canal were calculated. All intensity values were normalized as the ratio of the value for each ROI to the concurrent value for the reference solution.

The T2-weighted images were used to define the average brightness of the ROI. For each animal, the average intensity values of these regions were plotted as a function of time since TVPm injection to describe temporally any increases in bound (mucosal inflammation) and free water (effusion) within the ME. These images were also used to define the relative area of involvement with respect to inflammation and effusion. There, the number of pixels within the anterior petrosal and posterior mastoid ROIs whose intensities exceeded a specified threshold were counted and expressed as a percentage of the total number of pixels for that ROI by using the "masking" option of the system's software. This was done for two masking thresholds corresponding to 0.33 and 1.0 times the measured intensity of the reference solution.

Data for the sequential T1-weighted images obtained after bolus administration of the contrast agent in each scanning session were used to estimate the degree of vascular permeability. There, the rate of accumulation of contrast agent within the mucosa and effusion following intravascular administration was measured as the rate of change in intensity values for the specified ROIs. To quantify the rate, we calculated the difference between the average intensity value for the sequence most closely approximating a 20-minute postinfusion end point and that of the precontrast sequence. Greater values of this difference are indicative of higher rates of leakage between the vascular compartment and the ME.

For each animal, the temporal patterns for changes in these variables were compared with those for tympanometrically measured ME pressures.

RESULTS

None of the left ears of animals in cohort 2 developed abnormal ME pressures during the period of follow-up. In contrast, the right ears of the 10 animals showed one of three distinct patterns of change that

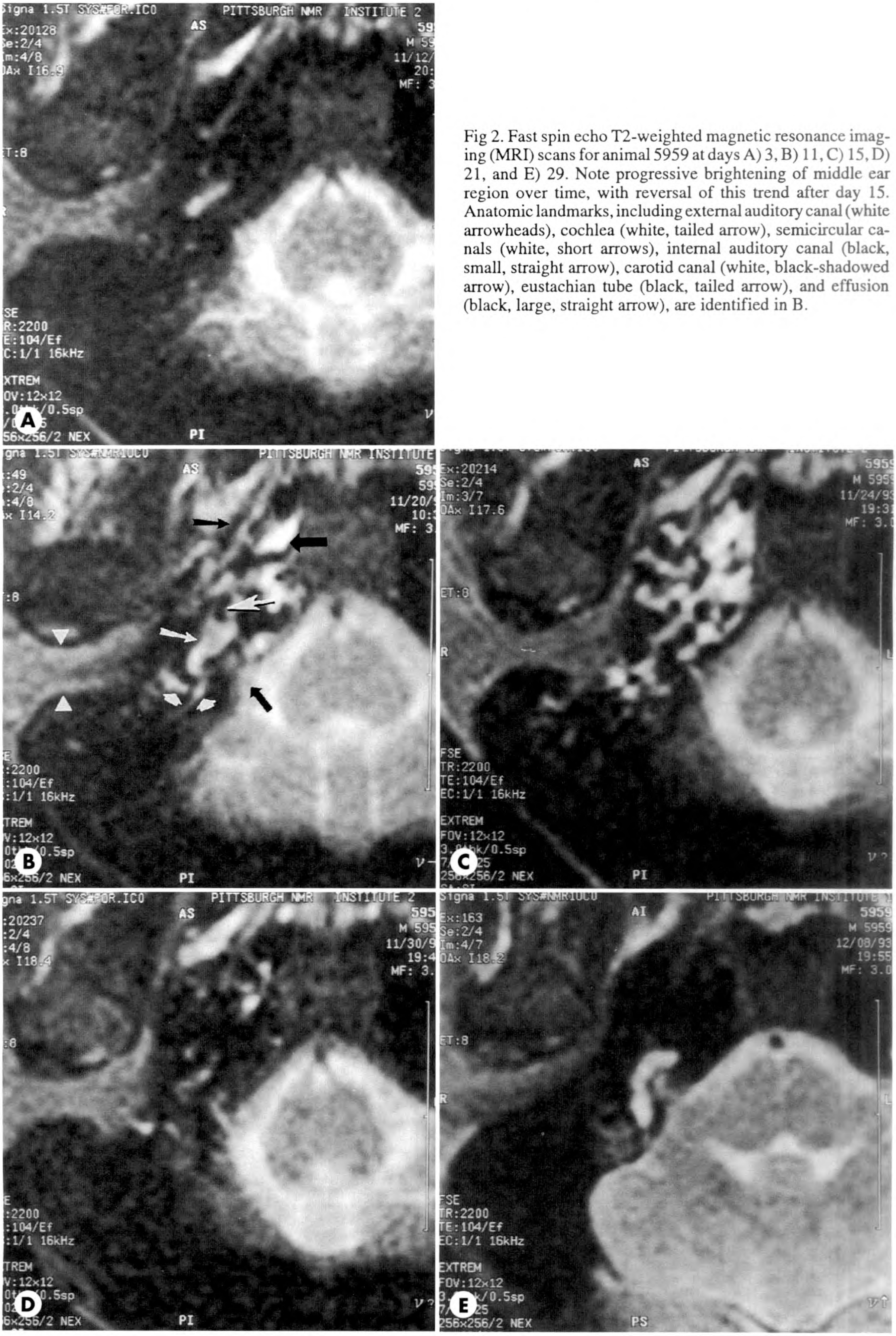

Fig 2. Fast spin echo T2-weighted magnetic resonance imaging (MRI) scans for animal 5959 at days A) 3, B) 11, C) 15, D) 21, and E) 29. Note progressive brightening of middle ear region over time, with reversal of this trend after day 15. Anatomic landmarks, including external auditory canal (white arrowheads), cochlea (white, tailed arrow), semicircular canals (white, short arrows), internal auditory canal (black, small, straight arrow), carotid canal (white, black-shadowed arrow), eustachian tube (black, tailed arrow), and effusion (black, large, straight arrow), are identified in B.

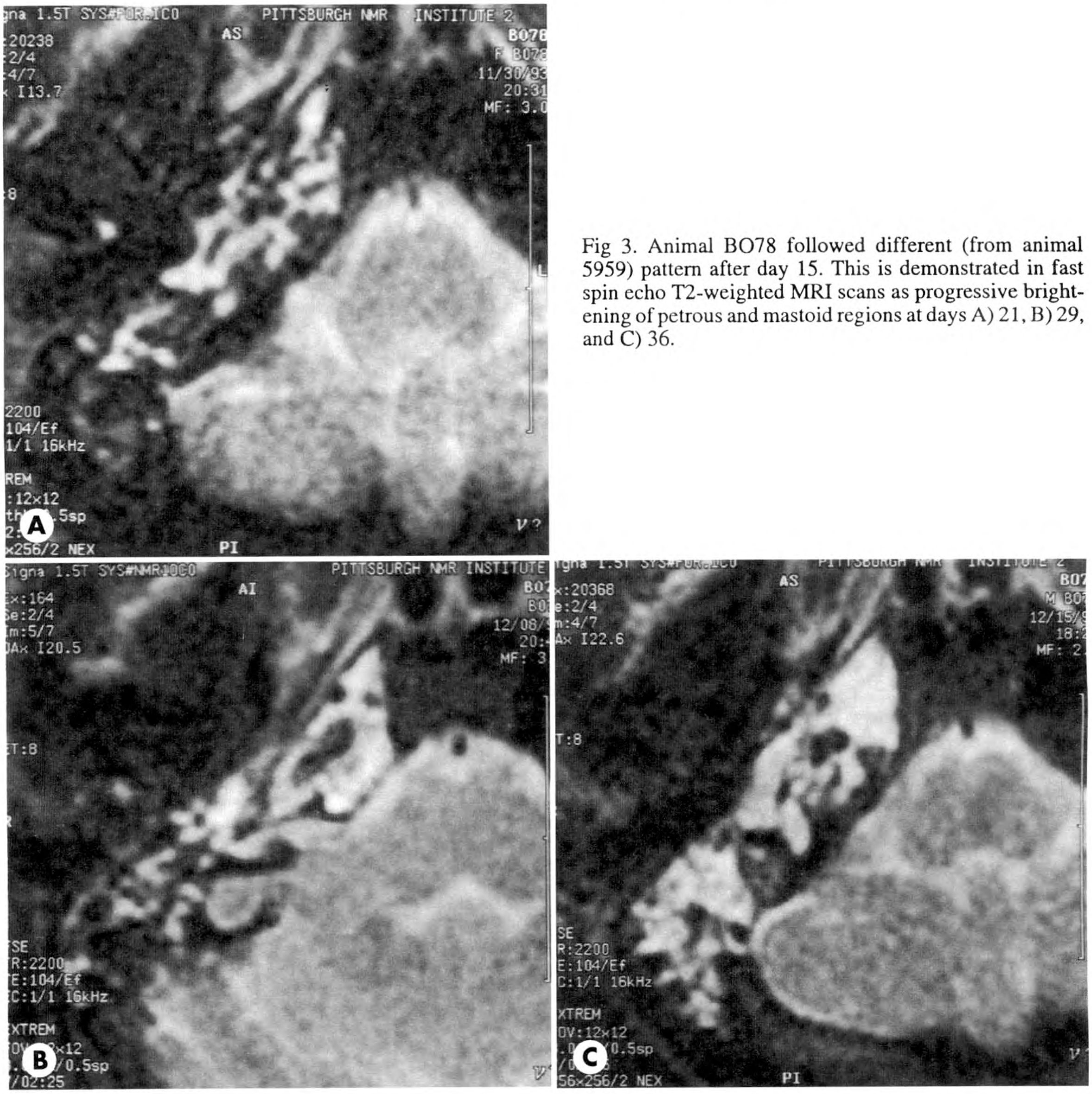

Fig 3. Animal BO78 followed different (from animal 5959) pattern after day 15. This is demonstrated in fast spin echo T2-weighted MRI scans as progressive brightening of petrous and mastoid regions at days A) 21, B) 29, and C) 36.

were labeled for reference as positive (n = 2), biphasic (n = 3), and negative (n = 5) profiles. The average daily ME pressures for the subset of ears showing each of these three response profiles are shown in Fig 1. The first profile was characterized by the development of positive ME pressures early after botulinum injection. These positive pressures were maintained for approximately 2 weeks and were followed by relatively normal ME pressures for the remainder of the study. For those 2 animals, right tympanic membrane compliances were relatively unchanged. The second profile was distinctly biphasic, characterized by an initial decrease in right ME pressures to approximately –400 mm H_2O by day 14 and then a recovery to more normal pressures by days 18, 26, and 28 in the 3 animals. The tympanic membrane compliance of these ears decreased and increased in phase with ME pressure. The third profile was characterized by the development of sustained negative ME pressures. Average ME pressure for these 5 ears decreased to less than –400 mm H_2O by postinjection day 14 and remained below that pressure threshold for the duration of follow-up. These changes in ME pressure were accompanied by a decrease in measured compliance to values that approached 0.

Visually, no changes in the T2-weighted images indicative of mucosal inflammation or effusion were observed at any time for the left ears of any of the animals or for the 2 right ears that exhibited a positive pressure profile. No change was observed over time in the image intensity of the ET lumen or mucosa of either ear of any animal. However, a progressive brightening of the petrous and mastoid ME ROIs without evidence of recovery during the study period was observed for the right ears of the 5 animals that

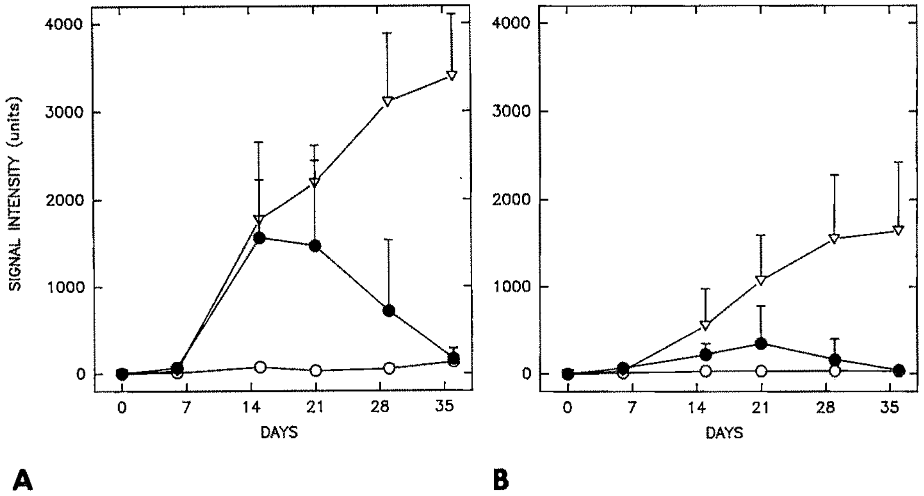

Fig 4. Average fast spin echo T2-weighted signal intensities as function of time for A) anterior and B) posterior regions of interest of right ears that failed to develop negative pressure (open circles), that developed and resolved negative pressure (closed circles), and that developed sustained negative pressure (open inverted triangles). Vertical bars — 1 SD about mean.

exhibited a negative ME pressure profile. The visual appreciation of brightness was increased and then decreased corresponding to the development and resolution of ME inflammation and effusion in the 3 right ears that exhibited a biphasic ME pressure profile. This pattern is exemplified in Fig 2, which shows the MRI scans of animal 5959 at selected times during the study. There, a progressive brightening of the tissue was observed in the area of the right ME, anterior air cells, and mastoid region on days 7, 11, and 15, with a reversal of this trend observed on day 21 and later. For reference, MRI scans of the right ear of an animal that displayed a negative ME pressure profile are shown for days 21, 29, and 36 in Fig 3, in which there is a progressive increase in the level of brightness in the area of the ME and associated air cells.

The visual changes in the MRI for each animal were quantified both as the average intensities for the anterior and posterior ROIs of the right ME and as the relative area of those regions that exceeded predefined intensity thresholds. The first measure is an index of the degree of inflammation, while the latter provides information as to the extent of involvement. Figure 4 shows the average intensities for the anterior and posterior ROIs of the right ME for subsets of animals displaying each of the three ME pressure profiles. In general, these data tracked well the temporal changes in ME pressure observed for the respective subsets. Specifically, increased average intensities of both the anterior and posterior air cells were associated temporally with periods of time when ME pressure was measured to be less than −300 mm H_2O. Figure 5 shows the average relative area of involvement as a function of time for subsets of right ears displaying each of the three ME pressure profiles based on masking thresholds set at 0.33 and 1.0 times the intensity value of the reference solution. Paired data for the two masking levels were similar within each subset, though as expected, the less stringent criterion was associated with a greater area of involvement. No inflammatory involvement was documented for the 2 ears that did not develop ME underpressures, while a progressively increasing area of involvement was documented for the 5 ears that

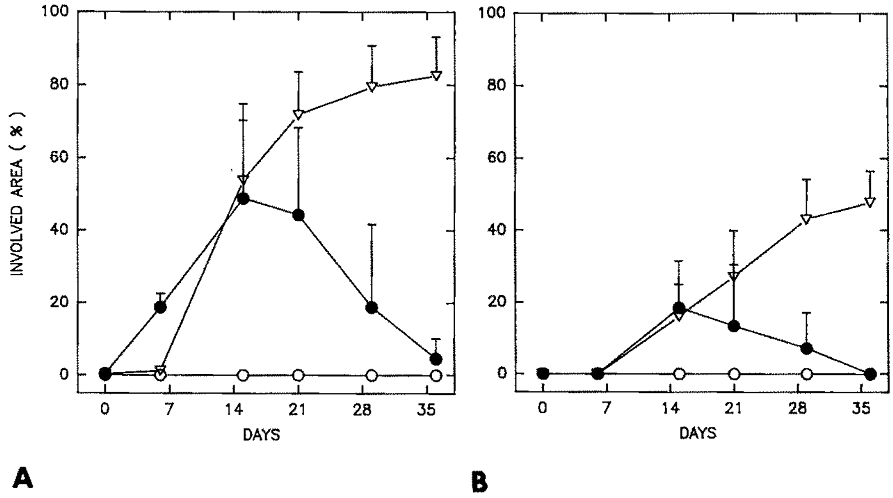

Fig 5. Average relative area of involvement at masking thresholds of A) 0.33 and B) 1.0 times reference intensity as function of time for right ears that failed to develop negative pressure (open circles), that developed and resolved negative pressure (closed circles), and that developed sustained negative pressure (open inverted triangles). Vertical bars — 1 SD about mean.

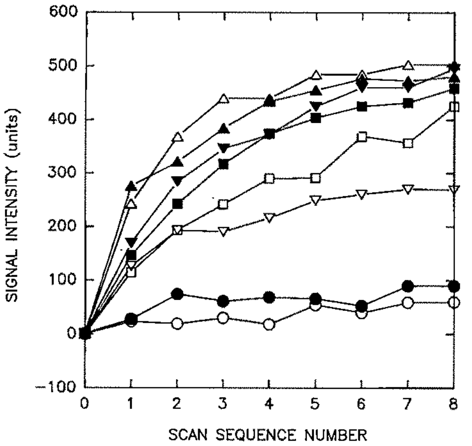

Fig 6. Signal intensity of sequential spin echo T1-weighted scans after gadopentetate dimeglumine injection for animal BO78 at days 0 (open circles), 3 (closed circles), 6 (open inverted triangles), 11 (closed inverted triangles), 15 (open squares), 21 (closed squares), 29 (open triangles), and 36 (closed triangles).

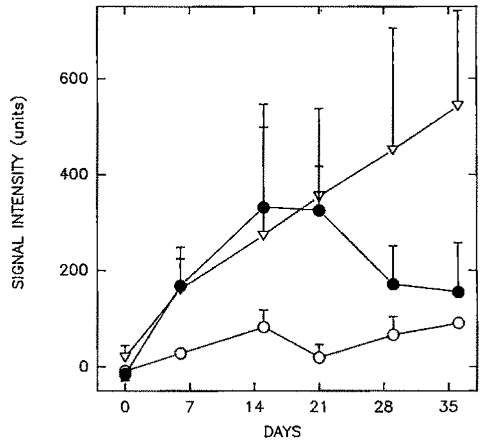

Fig 7. Average values for index of vascular permeability as function of time for right ears that failed to develop negative pressure (open circles), that developed and resolved negative pressure (closed circles), and that developed sustained negative pressure (open inverted triangles). Vertical bars — 1 SD about mean.

developed sustained underpressures. An increased and then decreased area of involvement was observed for the 3 ears with negative ME pressures that later resolved.

T1-weighted signal intensities of the ROIs recorded before and on repeated sequences following administration of the contrast agent gadopentetate dimeglumine were used to define a measure of vascular permeability. An example of these data is provided in Fig 6, which shows the T1 intensity values as a function of time since gadopentetate dimeglumine administration for the anterior region of the right ear of 1 animal in cohort 1 (BO78) at different scanning sessions. For all sessions, the function was curvilinear, with an asymptote parallel to the x-axis. Early sessions had asymptotic values of relatively low magnitude, but these increased for the later sessions, indicating increased vascular permeability. Because the asymptote was approached consistently by the sequence obtained 20 minutes after contrast administration, the intensity value for that sequence (referenced to the baseline) was used as a measure of the degree of vascular permeability. Figure 7 shows the average value of this index as a function of time for the anterior ROI of right ears in each of the three subsets defined on the basis of ME pressure response. In general, little change in this index was noted for ears that did not develop negative pressures, while the values showed a time-dependent increase for the 5 ears that developed sustained negative pressures. As with the measures of inflammation, the values of this index increased and then decreased for the 3 ears that developed and then resolved ME underpressures. There is noticeable mucosal enhancement of the fifth postcontrast image of animal 5959 on days 11 and 15 (Fig 8B,D) as compared with the images of the respective baseline sequences (Fig 8A,C). For each animal, a similar pattern of change was observed for the posterior ROI.

DISCUSSION

In an earlier study, injection of botulinum toxin into the TVPm of rhesus monkeys caused functional ET obstruction (ie, inability to dilate the ET by swallowing) as assessed by the forced-response and inflation-deflation tests.[20] Active ET function improved during the 4th week and was within normal limits by the 11th week postinjection. In those experiments, ME pressures decreased in 10 of 12 ears, all of which were diagnosed as developing effusions between day 8 and day 28 postinjection on the basis of tympanometric criteria or fluid recovery by tympanocentesis. However, no animals were sacrificed for histologic confirmation of disease. In the present study, that model was used in conjunction with MRI protocols to define the temporal relationships between changes in ME pressures, vascular permeability, and mucosal inflammation. Because more recent studies showed that bilateral botulinum injections of the TVPm caused severe distress in monkeys,[26] the procedure was limited to the right ear and the dose of botulinum toxin was reduced.

A number of past studies reported the diagnostic value of MRI with respect to inflammation, presence of free fluids, and degree of mucosal permeability.[24,25,27-32] Specifically, an earlier comparative study of T1-weighted MRI and histologic sections documented good correlations for various measurements of the ME mucosa.[33] Other studies showed that the intensities of T2-weighted images were much higher

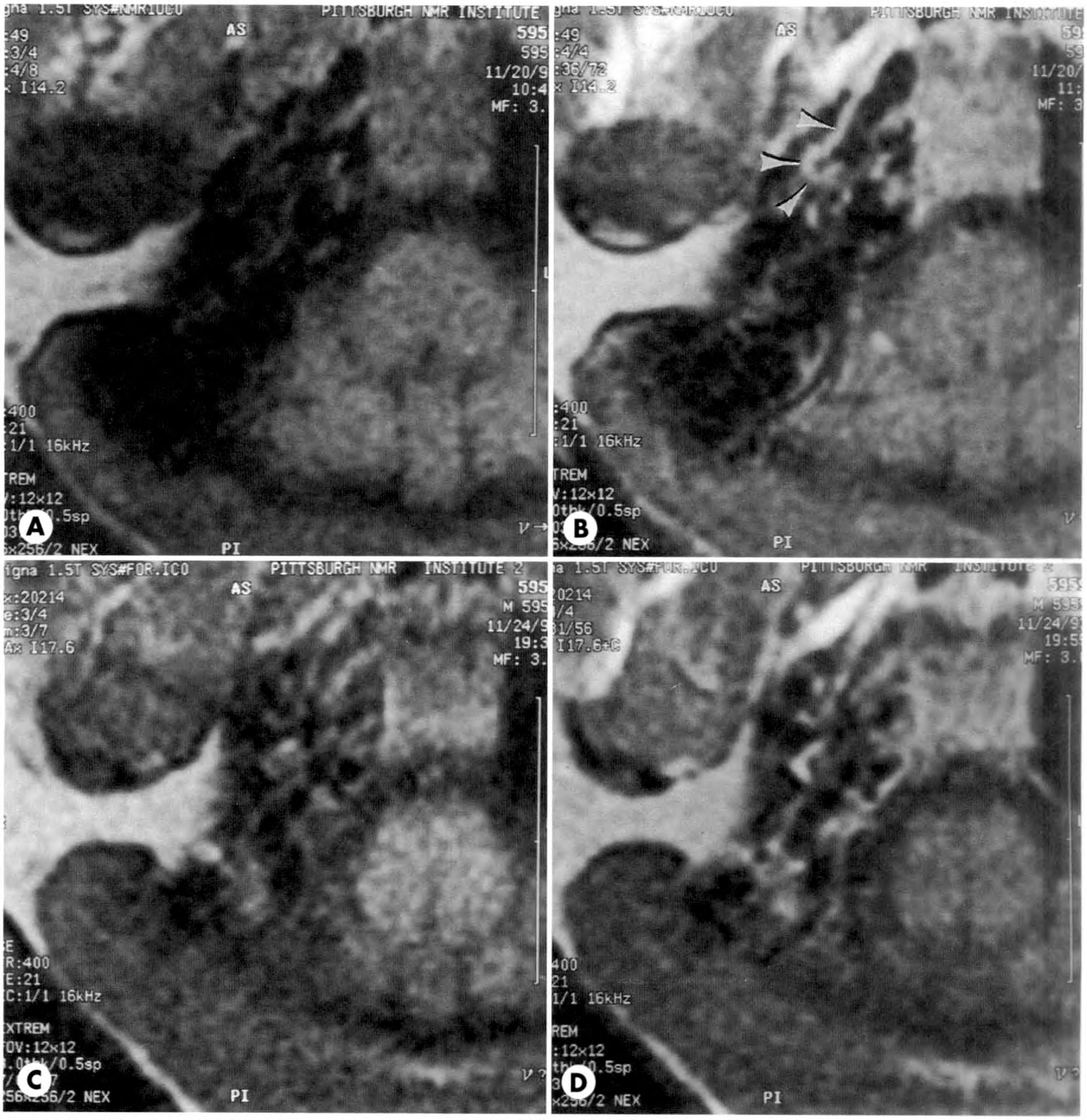

Fig 8. Spin echo T1-weighted MRI sequences of same animal as in Fig 2 (No. 5959). A) Precontrast and B) fifth postcontrast sequences demonstrate mucosal enhancement at day 11 (arrowheads). Increase in contrast enhancement with progression of inflammation is noticed in C) precontrast and D) fifth postcontrast sequences at day 15.

for inflamed tissues when compared to surrounding tissues.[24,27-32] For the ME and paranasal sinuses, mucosal inflammation and effusion were shown to have high T2-weighted MRI intensities, and demarcation of the boundary between the inflamed mucosa and free fluid was reported to be enhanced by introduction into the vascular compartment of the contrast agent gadopentetate dimeglumine.[25,29,31] Also, the degree of tissue enhancement following administration of gadopentetate dimeglumine was shown to provide a measure of the degree of inflammation.[25] Because this strongly hydrophilic agent does not easily permeate intact plasma membranes or cross an intact blood-brain barrier, the rates of accumulation of gadopentetate dimeglumine in mucosa and effusions have been used as measures of vascular permeability with respect to the mucosal interstitium and the epithelial barrier, respectively.[25] In the present study, these MRI protocols were adapted for use in the monkey.

The results of this study showed that injection of the TVPm with botulinum toxin resulted in a progressive decrease in ME pressures in 8 of 10 ears — comparable to the frequency of negative ME pressures (83%) reported previously for that procedure. However, unlike the results from the earlier study, a dosing effect was suggested by the higher frequency of effusions documented for the right ears of cohort 2, which were injected with weight-adjusted, individualized doses (100%), when compared to that for the right ears of cohort 1 (66%), which were given a standard dose of botulinum. Since botulinum paralyzes the TVPm, which is recognized as the sole tubal dilator in monkeys, gas exchange between the ME and environment via the ET is abolished. A functional isolation of the ME gas pocket from the environment is the prerequisite event for the development of OME by the hydrops ex vacuo mechanism. Therefore, this model is ideal for evaluating the validity of the component assumptions of that theory.

Following induction of ET obstruction, the first event predicted by the hydrops ex vacuo mechanism is the development of negative ME pressures caused by the gradient-driven ME-to-blood exchange of gas. In this experiment, negative pressures did not develop in the control ears, but did develop in 8 of the 10 right ears after injection of botulinum toxin. The failure of 2 right ears to develop negative pressures may have resulted from an incomplete paralysis of the TVPm, with the observed positive pressures generated by autoinflation as previously described for monkeys.[34] In contrast, the progressive development of negative ME pressures in the other 8 right ears most probably reflects the net loss of ME gases by exchange with the blood. This mechanism is consistent with the measured gas partial pressure gradients between ME and blood and with the predictions of general equations for gas exchange across a barrier. However, the relatively slow rate of pressure decrease observed in this study (approximately 40 mm H2O/24 h) is not consistent with the rate (350 mm H2O/24 h) predicted for monkeys by mathematic models that assume identical exchange limitations for the three physiological gases.[35] Recently, experimental measurement of the exchange rates for the different gases showed that nitrogen exchange is orders of magnitude slower than that predicted on the basis of the exchange rates of either oxygen or carbon dioxide under similar exchange regulations.[36] Those results and the fact that nitrogen is the major contributor to the total pressure gradient between ME and blood[37] suggest that the slow rate of ME pressure decrease documented in this experiment directly reflects the slow rate of transmucosal nitrogen exchange.

A second component of the hydrops ex vacuo theory is a negative pressure–induced increased permeability of the ME mucosa to fluid transfer. This event was confirmed by the data for the Tl spin echo sequences collected before and after bolus injection of gadopentetate dimeglumine that documented a progressive brightening of the sequential images for test sessions conducted during periods of negative ME pressures. The defined index of vascular permeability was not changed during the period of follow-up in the control ears or in the 2 right ears that did not develop negative pressures. However, increases in this index that tracked temporally negative ME pressures were observed for the other 8 right ears. Examination of the data for individual animals showed that changes in this index were associated with negative ME pressures of approximately –200 mm H2O and less. For the 3 right ears with biphasic ME pressures, the value of the index was not quickly normalized with the return to near-ambient ME pressures.

As predicted by the hydrops ex vacuo theory, negative ME pressures and increased mucosal vascular permeability preceded in time mucosal inflammation and effusion as assessed by the MRI. Unlike the paranasal sinuses and the rodent bulla, the monkey tympanum is small and communicates anteriorly and posteriorly with aerated regions composed of numerous septated air cells. This makes difficult the clear distinction between the presence of free (effusion) and bound (inflammation) water. Nonetheless, the increased MRI intensity noted for the ears that developed negative pressures confirms the accumulation of fluid in these air-containing spaces. The use of masking at specified thresholds showed that the distribution of effusion and inflammation was not localized to specific regions, but extended to most of the available volume of these spaces. A causal association between negative pressure and effusion is supported by the results for the 3 right ears that developed and resolved both conditions in phase, and by the lack of these changes on MRI for the 2 ears that failed to develop significant negative pressures and for the 4 control ears (left, cohort 2). A comparative examination of the data for individual animals of cohort 1 shows that 1) the presence of effusion was preceded by changes in the index of vascular permeability and 2) a negative ME pressure of approximately –300 mm H2O represents a critical threshold for the development of inflammation and effusion. Of interest is the rapid resolution of the effusion and inflammation in these ears within 1 week of the first observation of near-ambient ME pressures despite a retained high value for the index of vascular permeability.

The results of this study support the hydrops ex vacuo theory as a viable explanation for the development of ME effusions. They show that disrupting the normal function of the ET by paralysis of the dilatory musculature causes negative ME pressures. We suggest that these pressures result from the net loss of nitrogen by gradient-driven exchange with the blood, and not from the exchange of oxygen as has been hypothesized previously. The negative pressures in turn provoke changes in the permeability of the mucosal vasculature and, at a critical level of approximately −300 mm H_2O, effusion within the tympanum and associated air cells. Somewhat unexpectedly, the effusions, but not the changes in vascular permeability, resolved quickly with the reestablishment of more normal ME pressures. While these changes were initiated by a functional ET obstruction similar to that documented for children in the clinical setting, the role of this mechanism in the pathogenesis of OME in that population remains to be determined.

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IMAGING CASE STUDY OF THE MONTH

VALUE OF TECHNETIUM-99M PERTECHNETATE IMAGING IN THE DIFFERENTIAL DIAGNOSIS OF SALIVARY GLAND LESIONS

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INTRODUCTION

A neoplasm of the parotid gland arises as a "cold" area on a salivary gland scintigram, whatever the nature of the neoplasm, with the exception of a Warthin's tumor (papillary cystadenoma lymphomatosum) and some forms of oxyphilic adenomas. These latter tumors show increased uptake of radioiodine and technetium-99m pertechnetate ($^{99m}TcO_4^-$). Therefore, $^{99m}TcO_4^-$ imaging of the salivary glands can be of supportive value in the evaluation of salivary gland lesions, particularly of Warthin's tumors, as illustrated by the following case.

CASE REPORT

A 61-year-old man was investigated for a persistent swelling in the region of the right parotid gland that originated after an episode of infectious mononucleosis. Essentially, the mass was asymptomatic. The patient had complaints of shortness of breath, coughing, pain in the right shoulder, and symptoms of exercise-dependent retrosternal pain. He had smoked 25 cigarettes daily for more than 50 years. His medication included nifedipine, gliclazide, and acetylsalicylic acid. Physical examination revealed no head and neck abnormalities except for a mass in

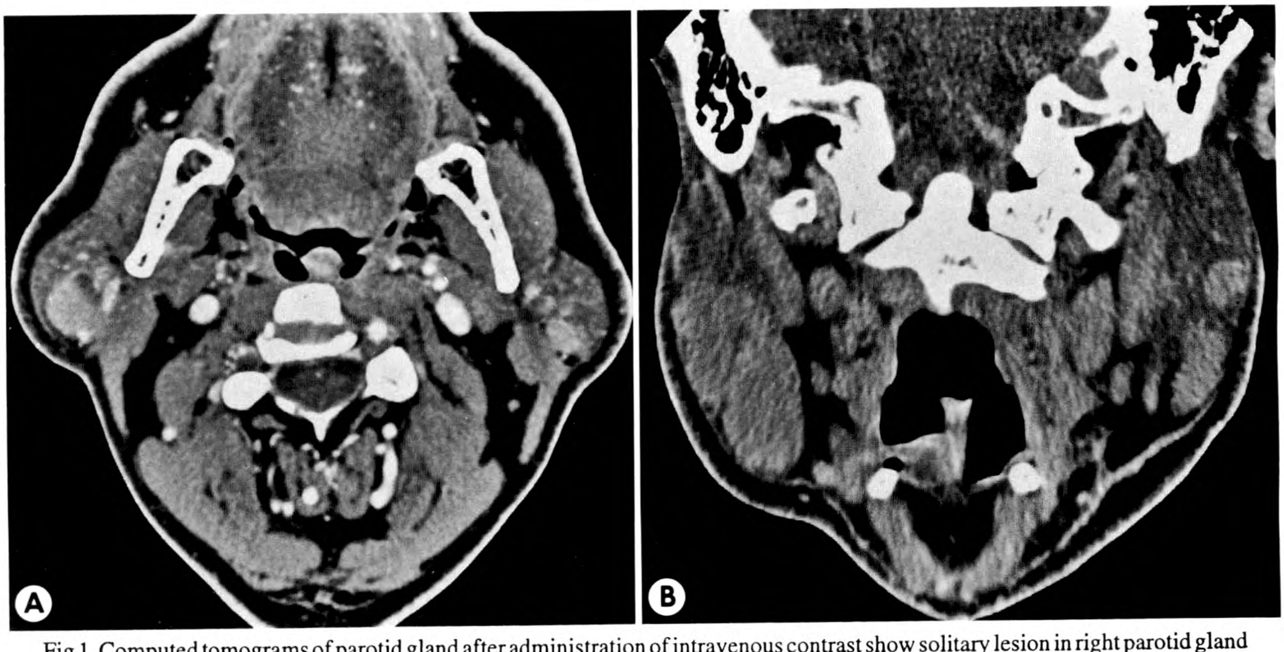

Fig 1. Computed tomograms of parotid gland after administration of intravenous contrast show solitary lesion in right parotid gland and two discrete lesions in left parotid gland. Tumors have smooth margins and are not infiltrating into surrounding tissues. A) Axial. B) Coronal.

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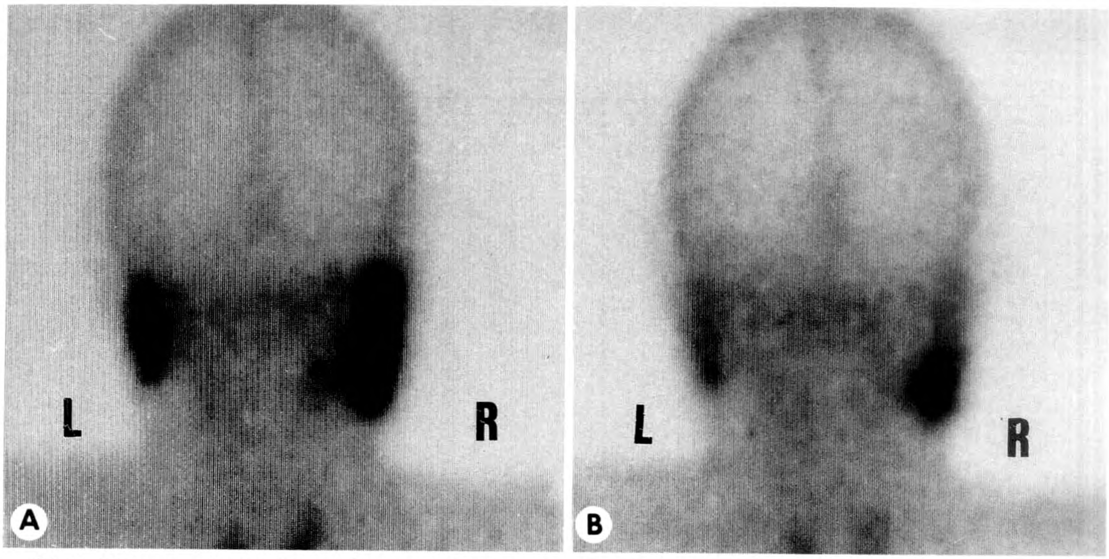

Fig 2. Right parotid gland shows intense uptake of technetium-99m pertechnetate on both A) predischarge and B) postdischarge (after lemon juice administration) scans. Scintigram of left parotid gland shows smaller lesion on both predischarge and postdischarge images, revealing bilateral disease.

the tail of the right parotid gland, 2 cm in diameter. There was no associated numbness in the area, and no facial weakness existed.

Computed tomography (CT) of the parotid gland region revealed a smoothly marginated mass in the posterior superficial lobe of the right parotid gland, and two smaller lesions in the posterior superficial lobe of the left parotid gland. There was no infiltration of the surrounding tissues (Fig 1). In addition, in the neck, bilaterally, several scattered lymph nodes were present, all smaller than 1 cm in diameter. Subsequently, salivary gland scintigraphy was performed. After an intravenous injection of 200 MBq $^{99m}TcO4^-$, data were acquired at 5-second intervals during the first minute and at 1-minute intervals during the next 15 minutes. These dynamic scintigraphic images of the parotid gland showed a lesion of intense uptake in the caudal area of the right parotid gland and, moreover, a smaller lesion of increased flow in the caudal pole of the left parotid gland. After administration of lemon juice (discharge test), static images at 5 minutes showed persistent increased uptake in both lesions, whereas the activity disappeared from the remaining part of both parotid glands (Fig 2). Fine needle aspiration cytology of the lesion in the right parotid gland showed normal parotid acini and a few oncocytic cells associated with numerous lymphoid cells, consistent with a Warthin's tumor.

Another incidental finding on a routine chest radiograph was a mass, measuring 1 cm in diameter in the upper lobe of the right lung, suggesting a primary carcinoma of the lung or metastatic disease. Two successive CT investigations showed an unchanged, hyperdense, well-restricted lesion, not connected to the pleura, in the upper lobe of the right lung, and some bilateral lymph nodes. Both tumor and lymph nodes were all smaller than 1 cm in diameter. However, because the patient refused further medical treatment, definitive histology of the lung tumor could not be obtained.

DISCUSSION

This study shows the benefit of salivary gland scintigraphy in the differentiation between benign and malignant parotid lesions. Warthin's tumors account for 2% to 10% of all salivary gland tumors and are seen generally in middle and old age. The tumor is bilateral in 5% to 10% of the cases, and often, multiple tumors are found in one or both glands. Mostly, the tumor is situated in the inferior part of the parotid gland.[1] As usual for parotid gland lesions, it was not possible to distinguish by CT alone whether a parotid tumor was benign or malignant.[2] When multiple lesions are seen in one parotid gland or bilaterally, the most likely diagnosis is Warthin's tumor. Moreover, cavitation is common.[3] Magnetic resonance imaging (MRI) is another increasingly important diagnostic tool for parotid gland lesions. However, as has been mentioned for CT, no definite differentiation can be made between benign and malignant salivary gland disorders by MRI alone, especially not for small, space-occupying lesions.[4] Increased $^{99m}TcO4^-$ uptake, and especially retained activity in washout images, is considered diagnostic of tumors that contain oncocytes (oncocytomas).[5] One of the most common of these tumors is Warthin's tumor, making a "hot" lesion quite indicative of Warthin's tumor.[6,7] In contrast to these benign tumors, metastatic lesions are always "cold."[5] Therefore, it can be concluded that if radiologic examination shows multinodular parotid gland lesions and a ^{99m}TcO4$^-$ scintigram reveals intense uptake on a postdischarge scan, a high probability for a Warthin's tumor exists. Although ^{99m}TcO4$^-$ scintigraphy has lost popularity in favor of fine needle aspiration, it is an effective diagnostic tool. Fine needle aspiration is a reliable and relatively safe diagnostic method when performed with proper technique. The accuracy rate varies from 87.5% to 97.9% in the literature. If a negative result does not conform to the clinical findings, the procedure must be repeated and a biopsy should be considered. Complications due to the procedure are rare and mostly minor.[8]

In this case, the combination of both a radionuclide scintigram and a positive fine needle aspiration of one lesion yielded a high probability of bilateral Warthin's tumor.

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IMAGING CASE STUDY OF THE MONTH

PREVERTEBRAL CALCIFICATION MASQUERADING AS A HYPOPHARYNGEAL FOREIGN BODY

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CASE REPORT

A 58-year-old woman presented to our emergency service with severe throat pain of sudden onset, incurred during a fish dinner. Physical examination of the hypopharynx and larynx was negative for localized trauma or a foreign body. A plain lateral neck radiograph was obtained, revealing a thin, elongated, opaque structure in the hypopharyngeal region compatible with a fish bone (Fig 1). As no foreign body was visualized despite its location as depicted in the neck radiograph, the patient was taken to the operating room and endoscopy was performed, followed by esophagoscopy, which were both negative. The procedure was terminated and the patient was brought into the recovery room. A follow-up radiograph revealed the same findings. Physical examination now revealed a small lesion on the lateral aspect of the hypopharynx with a raised, tunnel-like erosion extending from it inferiorly. Taking into account that this might have been iatrogenic, we sent the patient back to the operating room, and a full endoscopy and a local exploration of the traumatized area were performed, and again were negative. A repeat radiograph was no different, and the patient was taken for a computed tomography (CT) scan. Axial CT studies revealed a thin, round midline calcification (Fig 2) extending from C-3 to C-6. Because of the symmetry, midline location, and near-equal distance from the vertebral bodies, the finding was attributed to a ligament calcification and a "wait and see" approach was taken. On repeat questioning, the patient denied any former episode of possible bone or other foreign body

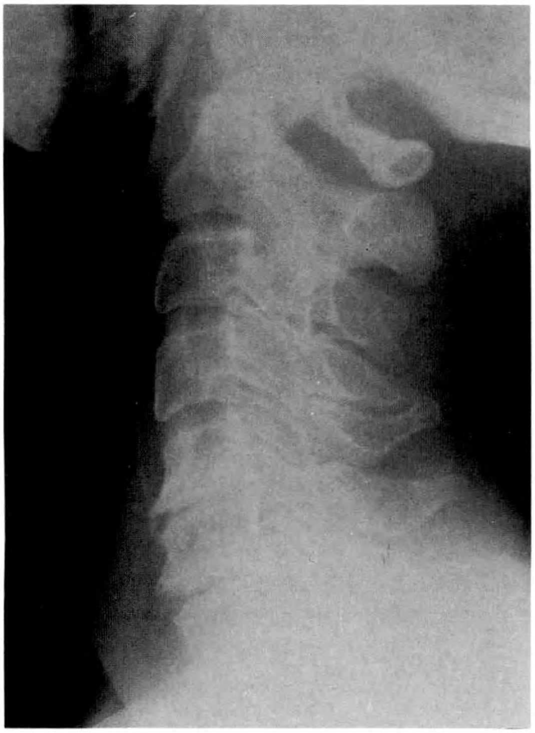

Fig 1. Lateral neck radiograph depicting thin, elongated, calcified vertical structure anterior to vertebrae.

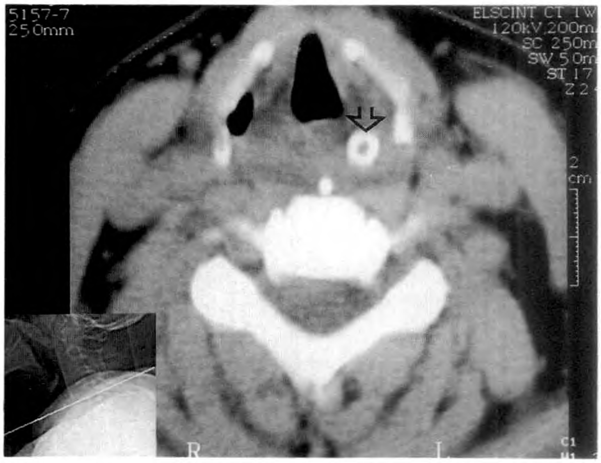

Fig 2. Axial computed tomogram showing calcification anterior to vertebral body. Arrow — feeding tube.

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ingestion. The patient was followed up for several days and was asymptomatic. She is now 6 months after the event with no complaints or adverse physical findings.

DISCUSSION

An unusual linear calcification of the posterior part of the cricoid cartilage was reported by Richardson[1] to simulate a foreign body. That patient underwent a second esophagoscopy after the radiologic findings persisted despite removal of a chicken bone in the first. This procedure was without any further findings. The issue of calcifications of the laryngeal cartilages was also discussed by Zoller and Bowie.[2] The authors state that normal calcifications of isolated portions of the thyroid, cricoid, arytenoid, and triticeous cartilages occur and are often imposed on the food passages, giving the impression of a foreign body. Goldman[3] describes his clinical experience in which 75% of fish bones eventually found were diagnosed by radiography. He further studied the radiopacity of bones of several fish species and found the majority to be opaque. Ell and Sprigg[4] also studied the radiopacity of bones of different fish species. Some of these were opaque, while others could barely be visualized despite their known position. This information is obviously valuable if the fish is of a species studied. This was not, however, the case in our patient.

The value of radiography in assessment of fish bone ingestion has been contested.[5] Seventy-one percent of bones eventually found by endoscopy were missed by the primary radiologist, while 29% were subsequently missed on review by two experienced radiologists. A case of a "false positive" was not described, however, in this series.[5]

We did not find any description of calcifications of the prevertebral tissues mimicking a foreign body. Despite the unusual circumstances of a highly reliable history and a highly suggestive radiograph, no foreign body of any kind was found in our patient. Anterior to the vertebral bodies lies the anterior longitudinal ligament, extending from the anterior arch of the atlas down to the sacrum. Anterior to it, in the midline, lies the posterior pharyngeal raphe, giving attachments to the pharyngeal constrictor muscles. This structure is attached above to the pharyngeal tubercle, extending to the lower part of the inferior constrictor muscle.

The suspected finding was seen on CT scan to be too symmetric in shape and location to be anything but a formerly unreported type of calcification, probably of the posterior pharyngeal raphe.

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CLINICOPATHOLOGICAL CONSULTATION

LYMPHOEPITHELIAL CARCINOMA OF THE LARYNX, HYPOPHARYNX, AND TRACHEA

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Lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea is a rare neoplasm composed of large, poorly differentiated, nonkeratinized cells intermingled with small nonneoplastic lymphocytes and plasma cells. It is histologically similar to its more common counterpart occurring in the nasopharynx. In contrast to nasopharyngeal carcinoma, most cases have not been associated with Epstein-Barr virus (EBV), although rare cases have been reported to be EBV-positive. The diagnosis often requires immunohistochemistry or electron microscopy for confirmation. The neoplasm seems to behave in a fashion reminiscent of nasopharyngeal carcinoma. Lymph node metastasis occurs in the majority of patients, and eventual visceral dissemination occurs in one fourth. Radiotherapy is the main treatment for the primary tumor and regional metastases, but chemotherapy is indicated for more advanced disease. The initial stage is the primary determinant of prognosis. Death from disease occurs in about one third of patients.

INTRODUCTION

Lymphoepithelial carcinoma is characterized by nests, sheets, or individual undifferentiated or poorly differentiated malignant epithelial cells surrounded and infiltrated by a prominent component of small, mature lymphocytes and plasma cells. The most frequent site of occurrence is the nasopharynx, where it is almost invariably associated with the Epstein-Barr virus (EBV).[1,2] It can also arise in a variety of sites, such as the sinonasal tract, nasolacrimal duct, oral cavity, oropharynx, salivary glands, thymus, hypopharynx, esophagus, stomach, larynx, trachea, lung, uterine cervix, vagina, breast, urinary bladder, and skin.[3-84]

Lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea is very rare, but has been recognized in the second edition[85] of the World Health Organization (WHO) *Histological Typing of Tumours of the Upper Respiratory Tract and Ear.*

TERMINOLOGY

The tumor has been denoted by a variety of terms, such as lymphoepithelial-like carcinoma, transitional carcinoma, undifferentiated carcinoma of nasopharyngeal type, undifferentiated carcinoma, anaplastic carcinoma with lymphoid component, undifferentiated carcinoma with lymphoid stroma, lymphoepithelioma, lymphoepithelioma of so-called Regaud type, lymphoepithelioma of so-called Schmincke type, and lymphoepithelial carcinoma. It is the last term, however, that has been most recently sanctioned by the WHO[85] and is currently preferred.

HISTORICAL PERSPECTIVE

Lymphoepithelial carcinoma was first reported in 1921 by Schmincke[58] in Germany and simultaneously but separately by Regaud and Reverchon[59] in France. The latter coined the term "lymphoepithelioma" to describe a particular undifferentiated carcinoma of the nasopharynx. Lymphoepithelial carcinoma has been subdivided into a Regaud type and a Schmincke type. In the former type, the neoplastic nonkeratinizing cells form cohesive nests embedded in a lymphoid stroma, and confusion with lymphoma is unlikely. In the latter type, there are isolated and dispersed nonkeratinizing cells scattered in the lymphoid tissue; the lesion is less easily identified as carcinoma and resembles a lymphoma. The two types have no prog-

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TABLE 1. LYMPHOEPITHELIAL CARCINOMA OF LARYNX, HYPOPHARYNX, AND TRACHEA

Authors	*Year*	*No. of Cases*	*Location*	*Demonstration of EBV*
Vossenberg[61]*	1928	1	Pyriform sinus	ND
Calvet et al[67]	1968	1	Larynx	ND
Dockerty et al[78]	1968	1	Hypopharynx	ND
Ferlito[79]	1977	1	Pyriform sinus	ND
Toker and Peterson[80]	1978	1	False vocal cord, ventricle	ND
Micheau et al[66]	1979	3	Ventricle, epiglottis Epiglottis Ventricle	ND
Stanley et al[50]	1985	4	True vocal cord, subglottis, trachea Epiglottis, base of tongue, vallecula Pyriform sinus, aryepiglottic fold False vocal cord, aryepiglottic fold, cricoid cartilage	ND
Friedmann and Ferlito[81]	1988	3†	Pyriform sinus	ND 2 cases, –ISH 1 case
Meyer-Breiting and Burkhardt[56]	1988	2	Larynx-hypopharynx	ND
Navarrete et al[55]	1989	1	Anterior commissure, subglottis	ND
Weiss et al[64]	1989	1	Pyriform sinus	–ISH
Onizuka et al[62]	1990	1	Trachea	ND
Wu et al[57]	1991	1	Larynx	–ISH
Caversaccio et al[84]	1995	1	Supraglottis, pyriform sinus	–Immunohistochemistry
Frank et al[69]	1995	1	Pyriform sinus	+ISH, +PCR, +ST
Andryk et al[70]	1996	1	Pyriform sinus	+ST
MacMillan et al[82]	1996	8	True and false vocal cords, ventricle (2 cases) Epiglottis (2 cases) Pyriform sinus (4 cases)	–ISH

EBV — Epstein-Barr virus, ND — not done (testing for EBV was not specifically performed), ISH — in situ hybridization, PCR — polymerase chain reaction, ST — serologic testing.

*Short communication regarding this case was presented in 1926 at ORL Congress in Hamburg by Marx.[60]

†One of these three cases was previously published by Ferlito.[79]

nostic difference.

LITERATURE REVIEW

Marx[60] briefly presented the first case of lymphoepithelial carcinoma originating in the pyriform sinus and involving the larynx in 1926 at the Otorhinolaryngologic Congress in Hamburg. Histologically, it was diagnosed as Schmincke type, and Schmincke personally reviewed the slides confirming the diagnosis. This case was widely reported and well documented histologically in 1928 by Vossenberg.[61] In 1968 Calvet et al[67] described a case of lymphoepithelial carcinoma with a Regaud pattern occurring in the larynx. In the same year, Dockerty et al[78] briefly reported another case of this tumor arising in the hypopharynx. Ferlito,[79] in 1977, observed an additional case of lymphoepithelial carcinoma of the left pyriform sinus presenting as a left cervical metastasis, after reviewing 2,052 cases of malignant laryngohypopharyngeal neoplasms. Toker and Peterson[80] described in 1978 a case involving the right false vocal cord and ventricle. In 1979, Micheau et al[66] found 3 more cases among 1,350 laryngeal carcinomas at the Institute Gustave-Roussy. Four additional cases were found at the Mayo Clinic during the years 1907 to 1984.[50] Three cases of lymphoepithelial carcinoma of the pyriform sinus encountered from January 1966 to December 1986 among 3,505 primary and secondary malignant tumors of the larynx and hypopharynx in the ENT Department of Padua University were mentioned by Friedmann and Ferlito,[81] and 1 of these 3 cases was subsequently evaluated for the presence of EBV by means of in situ hybridization for EBV early RNA (EBER-1), and it was negative. Of these 3 cases, 1 case was previously published.[79] Meyer-Breiting and Burkhardt[56] in 1988 reported 2 cases of lymphoepithelial carcinoma of the larynx and hypopharynx diagnosed in the ENT Department of Frankfurt University between 1962 and 1986. Navarrete et al,[55] in 1989, reported a case of lymphoepithelial carcinoma localized in the anterior commissure with invasion of the subglottic area. In the same year,

TABLE 2. REPORTED ASSOCIATION OF EPSTEIN-BARR VIRUS WITH BENIGN AND MALIGNANT DISEASES

Diseases	*Authors*
Infectious mononucleosis	Henle et al,[91] Derkay and Bramhall[92]
Lymphoid neoplasms	
Non-Hodgkin's lymphomas	Boyle et al,[95] Weiss and Chang[96]
Burkitt's lymphoma	Epstein et al,[86] Anwar et al[97]
T-cell lymphoma	Jones et al,[98] Su et al[99]
Hodgkin's disease	Weiss et al,[100] Tomita et al[101]
Lymphoepithelial carcinoma of nasopharynx	Zur Hausen et al,[106] Henle and Henle,[107] Wu et al,[57] Weiss et al[64]
Lymphoepithelial carcinoma of palate	To et al[13]
Lymphoepithelial carcinoma of tonsil	Brichacek et al,[6] Klijanienko et al[76]
Lymphoepithelial carcinoma of salivary glands	Saemundsen et al,[71] Hamilton-Dutoit et al,[49] Chan et al,[48] Kountakis et al[54]
Lymphoepithelial carcinoma of thymus*	Leyvraz et al,[75] Dimery et al,[47] Fujii et al[22]
Lymphoepithelial carcinoma of pyriform sinus	Frank et al[69]
Lymphoepithelial carcinoma of esophagus	Mori et al[63]
Lymphoepithelial carcinoma of stomach†	Burke et al,[4] Min et al,[45] Pittaluga et al,[17] Tokunaga et al,[73] Mori et al[63]
Lymphoepithelial carcinoma of lung	Bégin et al,[72] Butler et al,[46] Gal et al,[10] Pittaluga et al,[11] Chan et al[68]
Sinonasal carcinomas	Lopategui et al,[44] Gallo et al,[108] Leung et al[109]
Oral hairy leukoplakia	Greenspan et al,[93] Gilligan et al[94]
Basaloid squamous cell carcinoma of nasopharynx	Wan et al[110]
Smooth muscle tumors	Lee et al,[103] McClain et al[104]
Inflammatory pseudotumor of liver and spleen	Arber et al[105]

Lymphoepithelial carcinomas of larynx, skin, uterine cervix, breast, and urinary bladder were examined for EBV genomes by ISH and PCR and all were negative. Rare examples of lymphoepithelial carcinomas of nasolacrimal duct, trachea, and vagina were not examined for EBV genomes.

*EBV positivity for Southern blot hybridization has been reported in 2 noninvasive thymomas and 3 cases of thymic hyperplasia.[8]

†EBV has also been associated with adenocarcinoma of stomach.[102]

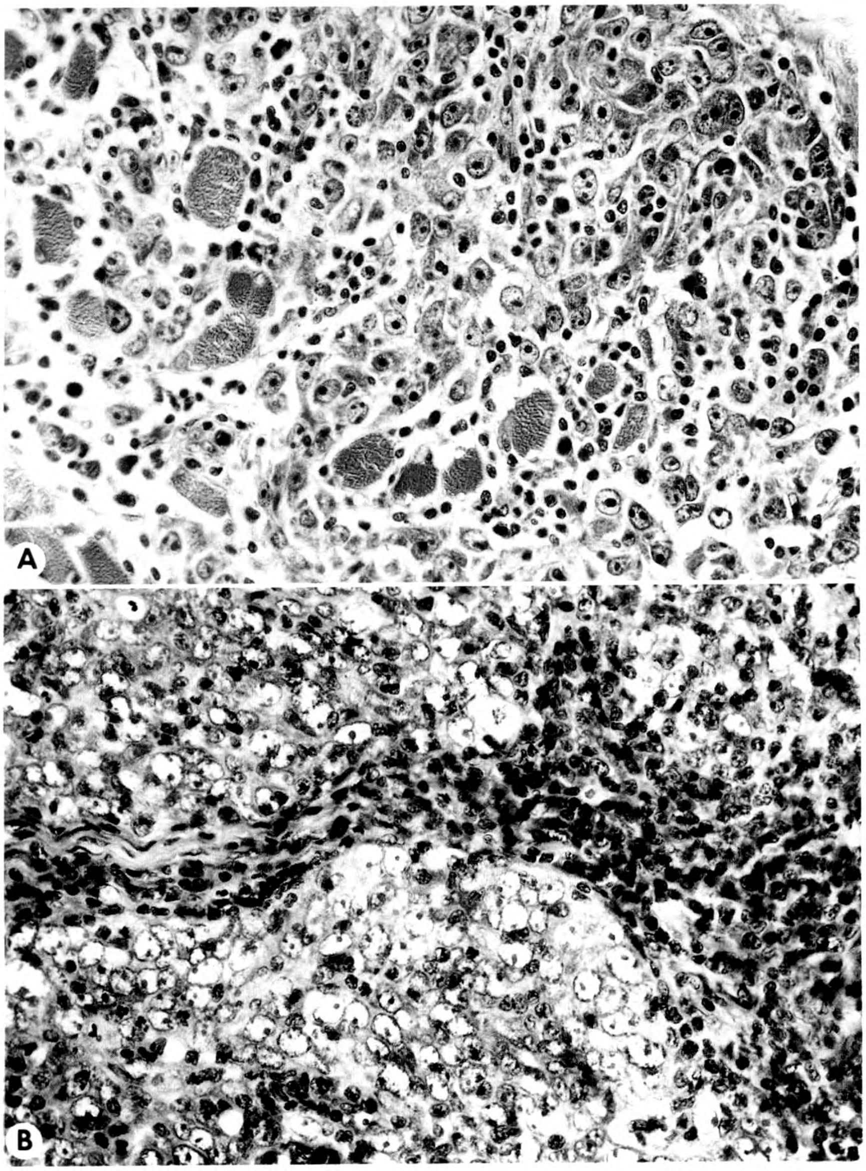
Weiss et al[64] mentioned a case of lymphoepithelial carcinoma of the pyriform sinus in which the results of in situ hybridization for EBV genomes were negative. In 1990, Onizuka et al[62] reported the first case of lymphoepithelial carcinoma involving the trachea. In 1991, Wu et al[57] mentioned a case of laryngeal lymphoepithelial carcinoma in which hybridization for EBV was not seen in either the malignant epithelial cells or normal tissues. Another case of lymphoepithelial carcinoma of the laryngohypopharynx was described by Caversaccio et al.[84] In 1995, Frank et al[69] described a case of lymphoepithelial carcinoma of the pyriform sinus and demonstrated an association of this lesion with EBV infection. This is the first reported case demonstrating this association, documented via in situ tumor DNA amplification (using the polymerase chain reaction) and hybridization techniques, as well as serologic testing. In 1996, Andryk et al[70] reported a case of lymphoepithelial carcinoma of the pyriform sinus associated with an elevated EBV titer.

Recently, MacMillan et al[82] reported 8 new cases of lymphoepithelial carcinoma of the larynx and hypopharynx accumulated over a period of 25 years at the Presbyterian-University Hospital and the Eye and Ear Institute, Pittsburgh, Pa. All 8 tumors exhibited alterations in p53 expression, but none was positive for EBV.

Table 1[50,55-57,60-62,64,66,67,69,70,78-82,84] summarizes all cases of lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea reported in the literature. None of the reported tumors have been metastatic to the laryngohypopharyngotracheal regions.

VIROLOGY

There is a strong evidence implicating EBV in the pathogenesis of a wide variety of neoplasms. The virus was originally discovered in 1964 by Epstein et al[86] in electron micrographs of cultured Burkitt's lymphoma tumor cells. A variety of techniques have been employed to demonstrate an association of EBV with benign and malignant diseases, including serology, polymerase chain reaction, Southern blot, in situ hybridization, and immunohistochemical studies. Serologic studies for EBV have been used as screening methods for the detection of nasopharyngeal carcinoma in large populations, but are of limited utility in investigating individual cases. Polymerase chain reaction studies are also of limited utility due to their exquisite sensitivity and the fact that EBV-seropositive individuals continue to carry EBV in a subset of their lymphoid cells. Southern blot studies are relatively insensitive, but one can determine the clonality of cells harboring EBV using a probe that hybridizes adjacent to the EBV genome terminal repeat region.[87] The most sensitive and specific test for the detection of EBV within tumor (and other cells) is RNA in situ hybridization for the EBER gene, a



Lymphoepithelial carcinomas of pyriform sinus (H & E, original ×100). A) Case showing muscle invasion. Neoplastic cells show large, usually centrally located, prominent nucleoli. B) Another case, showing cohesive nest of cells surrounded by prominent stromal lymphocytes. Neoplastic cells have uniformly vesicular nuclei and indistinct cytoplasmic borders.

region of the EBV that is amplified up to 10^6 times in latently infected cells.[88] Paraffin and/or frozen section immunohistochemical studies can be used to determine the presence of EBV-associated proteins such as latent membrane protein (LMP) and the Epstein-Barr nuclear antigens (EBNAs).[89,90]

With the above methods, EBV has been found to be the etiologic agent of many cases of acute infectious mononucleosis and acquired immunodeficiency syndrome–associated oral leukoplakia.[91-94] In addition, EBV has been associated with a wide variety of lymphoid proliferations and neoplasms, including a significant subset of Hodgkin's disease, endemic Burkitt's lymphoma, immunodeficiency-associated lymphoproliferations, and nasal T-cell lymphoma.[86,95-101]

Nasopharyngeal carcinoma is the epithelial neoplasm most consistently associated with EBV.[1,2]

However, a variety of foregut lymphoepithelial carcinomas have been associated with EBV, including salivary gland, lung, sinonasal region, stomach, thymus, palate, tonsil, pyriform sinus, and esophagus.[83] In the salivary gland, lung, and sinonasal region, the association with EBV appears to be restricted to Asian patients, whereas the association of EBV with gastric and thymic lymphoepithelial carcinomas appears to be independent of race or geography. In addition, a small subset of typical gastric adenocarcinomas lacking intense lymphocytic infiltration is also apparently associated with EBV.[102] Finally, occasional sarcomas, particularly smooth muscle neoplasms occurring in immunosuppressed patients, and stromal cells in some cases of inflammatory pseudotumor involving the liver and spleen, have been associated with EBV.[103-105]

Table 2[4,6,8,10,11,13,17,22,44-49,54,57,63,64,68,69,71-73,75,76,86,91-110] summarizes some of the benign and malignant EBV-associated diseases. The role of EBV in the induction of lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea is still unresolved. Initial results suggest that the EBV has a limited role, if any, in the causation of this tumor in patients of non-Asian descent. Whether this applies to similar tumors in patients of Asian origin is unknown.[82]

Of the 31 cases of lymphoepithelial carcinoma reported in the larynx, hypopharynx, and trachea, 14 have been specifically evaluated for the presence of EBV, and of these, only 2, both occurring in the pyriform sinus, have been claimed to be positive for EBV.[69,70] The association of EBV with the tumor was demonstrated by means of polymerase chain reaction, in situ DNA hybridization, and serologic testing in the first case. In the second case, there were elevated EBV titers — only indirect evidence that EBV was actually associated with the tumor.

PATHOLOGY

Macroscopically, lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea has no characteristic appearance. The lesion may even be polypoid. Microscopically, the neoplasm is composed of large, poorly differentiated, nonkeratinized cells with large, round, vesicular, sometimes "water-clear" nuclei, each containing a single large, usually centrally located, basophilic to deep red prominent nucleolus (see Figure, A). The cytoplasm is poorly defined and varies from amphophilic to slightly eosinophilic. Syncytial aggregates are often present. As a rule, there is a little fibrous tissue present. The neoplastic cells are intermingled with small, mature, nonneoplastic lymphocytes and, occasionally, plasma cells (see Figure, B).

Ultrastructural studies have not been reported for lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea.

Immunohistochemical stains for keratin and epithelial membrane antigen are positive, while negative staining for leukocyte common antigen among the tumor cells constitutes further proof of epithelial differentiation.[111] S-100 protein–positive dendritic cells are present, scattered among the tumor cells. Both B- and T-lymphocytes are identified in the lymphoid stroma with appropriate immunohistochemical stains.

DIAGNOSIS AND PRETREATMENT EVALUATION

The diagnosis of lymphoepithelial carcinoma is not difficult for the pathologist if the biopsy fragment is representative. This tumor has to be distinguished from lymphoma, and immunostaining or electron microscopic studies may be necessary in difficult cases. Other tumors that should be mentioned in the context of differential diagnosis are poorly differentiated squamous cell carcinoma, basaloid squamous cell carcinoma, and high-grade mucoepidermoid carcinoma in which a significant degree of lymphoid infiltration is present. Tumor staging is mandatory for therapeutic and prognostic reasons, and examination should include panendoscopy, chest radiographs, whole lung tomography, bone scintigraphy, neck and liver echography, and laboratory investigations.

TREATMENT

Although most patients have been treated surgically, lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea seems to behave in a fashion reminiscent of nasopharyngeal carcinoma, and should probably be treated similarly.[69] The tumor is radioresponsive, and radiotherapy is the main treatment. Radiotherapy should be limited to the primary tumor, or to the primary tumor and regional metastases. For more advanced and disseminated disease, adjunctive chemotherapeutic modalities are indicated.

PROGNOSIS

Lymphoepithelial carcinoma of the larynx, hypopharynx, and trachea is a rare, rather aggressive neoplasm with a propensity for early cervical lymph node metastasis in three fourths of cases, eventual visceral dissemination (lung, liver, bone, skin, and mediastinal and retroperitoneal lymph nodes) in one fourth, and death from disease in one third.[82] The prognosis is related mainly to the stage of the neoplasm at the time of the commencement of the therapy. Alterations in p53 expression are common in this neoplasm, but of no prognostic value.[82]

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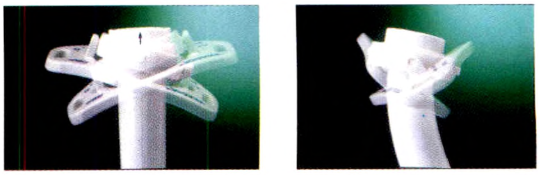

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PROGNOSTIC SIGNIFICANCE OF BCL-2 EXPRESSION IN LOCALIZED SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

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This study was conducted to determine whether Bcl-2 overexpression in localized squamous cell carcinoma of the head and neck (SCCHN) might serve as a marker for tumors unlikely to respond to standard treatment. Tissue samples from 33 patients undergoing surgery or irradiation for early-stage SCCHN during the years 1977 to 1992 were stained for Bcl-2. All patients had either T1N0 lesions of the oral cavity, pharynx, or larynx or T1N0 or T2N0 lesions of the true vocal cords. Of the 33 patients, 26 remained disease-free after at least 3 years of follow-up; the remaining 7 patients developed either tumor recurrence or a second primary tumor, 4 of which were fatal. Twelve patients had tissue specimens staining positive for Bcl-2; 6 of these patients had a poor outcome, and 6 had a good outcome. The relationship between poor outcome and overexpression of Bcl-2 in tumor cells was statistically significant ($p = .0047$ by Fisher's exact test). For tumors overexpressing Bcl-2, there was no significant difference in recurrence rate between those undergoing surgery and those undergoing radiotherapy as the primary mode of treatment. The overexpression of Bcl-2 in early lesions in this study predicted a cure rate of 50%, as opposed to the generally expected 90%, suggesting that Bcl-2 is a significant prognostic indicator in early SCCHN. Future studies will determine if altering the treatment will improve outcome in these patients.

KEY WORDS — Bcl-2, head and neck, prognosis, squamous cell carcinoma.

INTRODUCTION

Early squamous cell carcinoma of the head and neck (SCCHN) can be treated with either irradiation or surgery and has an excellent prognosis. Regardless of the type of treatment used, however, at least 10% of patients suffer poor outcomes, succumbing to their primary tumor, a recurrent tumor, or a second primary. It has long been a goal of cancer research to identify this subgroup of patients before treatment, so that protocols can be adjusted to improve their survival. According to one theory, the overexpression of certain gene markers may serve as an indicator of patients with a poor prognosis. So far, however, no such marker has been identified.

An important recent conceptual advance in cancer biology is the discovery that disorders of genes regulating programmed cell death (apoptosis) have significance comparable to disorders of genes regulating cell proliferation.[1,2] Furthermore, it is now clear that the gene products regulating cell proliferation and apoptosis are intimately interrelated in a complex network.[1] Apoptosis in cancer has recently assumed even more importance with the realization that it is possibly the principal mechanism by which irradiation and chemotherapy kill cancer cells.[3]

The role of the Bcl-2 gene as the primary regulator of apoptosis rests on abundant experimental data.[4] (The term Bcl-2 is an acronym for B-cell lymphoma/leukemia–2 gene.) Bcl-2 expression is essential for protecting normal cells from apoptosis, for example, during lymphocyte development.[2] Bcl-2 oncoproteins are found in the nuclear envelope, mitochondrial membrane, and endoplasmic reticulum.[5]

The importance of abnormalities of Bcl-2 gene structure or expression in cancer biology is revealed by their high frequency of occurrence in many human malignancies.[4] The well-defined structural abnormality (translocation t[14;18]) of the Bcl-2 gene that led to its discovery is confined to follicular B-cell lymphomas. The mechanism of deregulation in other tumors is under intense investigation, but is largely unknown. Investigation of the distribution of the Bcl-2 protein in normal and malignant tissues has been considerably assisted by the availability of monoclonal antibodies able to recognize Bcl-2 protein in routinely fixed tissue specimens.[6] This had led to demonstration of Bcl-2 oncoproteins in a variety of normal tissues, including lymphoid tissue, bronchial epithelium, basal cells of the skin, crypt cells of the intestine, breast, prostate, thyroid, and nasopharynx.[7]

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TABLE 1. SCALE FOR SCORING TUMOR STAINING

Bcl-2–Positive Cells	*Score*
<1%	0
1% - 10%	1+
11% - 35%	2+
36% - 70%	3+
>70%	4+

TABLE 2. TREATMENT MODALITY FOR 33 PATIENTS WITH LOCALIZED SQUAMOUS CELL CARCINOMA OF HEAD AND NECK

Treatment	*True Vocal Cord*	*Other Sites*
Surgical resection	15*	10
Radiotherapy	6	2

*Thirteen hemilaryngectomies and two laser cordectomies.

Normally, however, Bcl-2 proteins are found only in the basal or proliferating cells.[8]

Surprisingly, the biologic significance of Bcl-2 overexpression in tumors has varied widely in different reports.[9-11] It appears that the significance of Bcl-2 overexpression may depend, in part, on the biology of the individual tumor or cell of origin.

Few studies of Bcl-2 abnormalities in SCCHN have been reported. Although the prognosis of SCCHN is related to the site, size, and stage of the tumor, some seemingly early-stage tumors develop recurrence after treatment. It is not clear why some patients with these apparently curable tumors do poorly, and achieving a better understanding of their biology appears to be a prerequisite to significantly improving therapy for this disease.

The objective of this study was to determine the prevalence of Bcl-2 overexpression in localized SCCHN, and to investigate our hypothesis that it might be a marker for tumors unlikely to respond adequately to standard treatment. We selected early-stage tumors because they generally do respond well to one appropriately chosen mode of treatment, and cure can usually be achieved. It is particularly important to develop a method to identify patients who will not respond favorably in order to avoid undertreatment.

PATIENTS AND METHODS

Patients. The records of patients with SCCHN who were treated by the senior authors (M.F., D.D.C.) during the years 1977 to 1992 were reviewed. A criterion for inclusion in this study was that the lesions be early-stage: T1N0 lesions of the oral cavity, pharynx, or larynx, or T1N0 or T2N0 lesions of the true vocal cords. Patients were included only if they had posttreatment follow-up of at least 3 years or died of the tumor within 3 years after treatment. The Bcl-2 staining procedure for this study required paraffin tissue blocks containing well-preserved areas of tumor. All eligible patients for whom appropriate tissue blocks and clinical follow-up were available were included in the study. In addition, because of the possibility that apparently normal epithelium from tumor-bearing patients who smoked might not be truly normal because of intensive carcinogen exposure, archival tissue from 20 nonsmoking patients undergoing uvulopalatopharyngoplasty for relief of snoring was also obtained. These specimens served as a negative control.

A poor treatment outcome was defined as one of the following: 1) tumor recurrence, either local or distant, 2) a second primary tumor of the aerodigestive tract, or 3) death from tumor.

Method for Measuring Bcl-2 Expression. In preparation for antibody staining, paraffin-embedded head and neck tumor sections (5 µm in width) were deparaffinized and rehydrated by a standard technique and subjected to a microwave antigen retrieval procedure for 48 minutes in citrate buffer.[12] The tissue was stained on the Ventana ES320 Histo-stainer by means of supplied diaminobenzidine and avidin-biotin conjugate immunoperoxidase chemistry (Ventana Medical Systems, Tucson, Ariz[13,14]). Tissue sections were selectively stained at a 1:40 concentration for the Bcl-2 oncoprotein with the monoclonal mouse anti human Bcl-2 oncoprotein antibody (clone 124), supplied by DAKO Corp, Carpinteria, Calif (Ab: code M0887, lot 075). The morphology of sections stained for Bcl-2 was evaluated with the aid of hematoxylin counterstain.

Results of Bcl-2 tumor staining were scored visually on a scale of 0 to 4+ (Table 1). Any score above 0 was considered to represent overexpression. Normal small lymphocytes, present in every tumor section, served as an internal positive control for Bcl-2 staining.

Statistical Analysis. The possible associations between Bcl-2 overexpression, treatment outcome, and other categorical variables were evaluated with Fisher's exact test. Association of continuous variables was evaluated with a *t*-test. The log-rank test was done to compare survival among groups of patients. Survival curves were generated by the Kaplan-Meier method. A significance level of .05 was used for all statistical tests.

RESULTS

Thirty-three patient records and pathology specimens were included in the study. There were 28 men and 5 women, with a mean age of 59 years (range 35

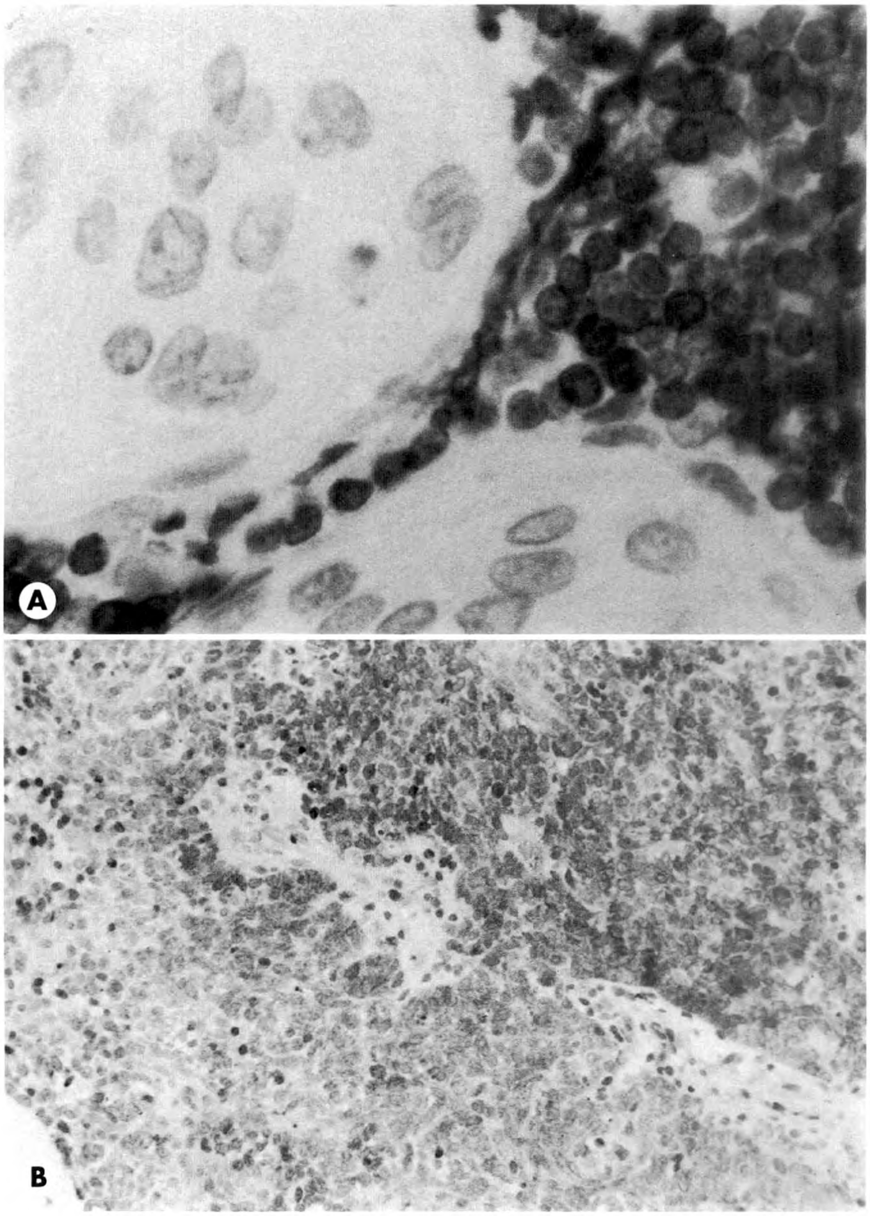

Fig 1. Photomicrographs of squamous cell carcinoma. A) Staining negative for Bcl-2. Note normal lymphocytes staining positive for Bcl-2 at top right. B) Demonstrating overexpression of Bcl-2.

to 87 years). Thirty of the patients were cigarette smokers. Twenty-one patients had carcinoma of the true vocal cord, with the remaining 12 patients having tumors of other sites in the head and neck (false vocal cord 2, floor of mouth 4, oral tongue 4, epiglottis 1, and soft palate 1). The primary modes of treatment are shown in Table 2.

Seven patients have died since treatment: 4 died of the squamous cell carcinoma and 3 died of causes unrelated to the tumor. The mean follow-up time for the remaining 26 patients was 91 months (range 36 to 220 months).

Seven patients in this series had a poor outcome. Six patients developed tumor recurrence, and 3 of these died of their tumor. One patient developed a second primary SCCHN that ultimately proved to be fatal.

Twelve (36%) of the 33 tumors were found to show significant overexpression of Bcl-2 protein (a score of 1+ to 4+). The well-differentiated SCCHNs overexpressing Bcl-2 showed staining in all levels of the malignant epithelium (Fig 1). Less well-differentiated tumors also showed staining in an allover or apparently randomly scattered distribution. The Bcl-2 staining showed a granular cytoplasmic pattern, with some accentuation around the nuclear membrane, as previously described.[15] In contrast, the archival control tissue from nonsmokers showed staining of only rare (<1%) squamous cells, all of which were in a basal location.

There was no relationship between the ages of the patients and Bcl-2 staining (p = .76 by two-sample *t*-

TABLE 3. OUTCOME ACCORDING TO TUMOR SITE AND BCL-2 STATUS

		Bcl-2 Overexpressed		*Bcl-2 Undetectable*	
Site of Tumor	*No.*	*Poor Outcome*	*Good Outcome*	*Poor Outcome*	*Good Outcome*
True vocal cord	21	2	5	0	14
Other sites	12	4	1	1	6
All sites	33	6	6	1	20

Poor outcome is defined as either tumor recurrence, second primary tumor, or death from tumor.

test). Nearly all of the patients gave a history of smoking, so it was not possible to analyze the relationship between smoking history and Bcl-2 overexpression in the tumor. Seven (33%) of the 21 true vocal cord tumors and 5 (42%) of the 12 tumors from other sites overexpressed Bcl-2. Bcl-2 staining was undetectable in the remaining 21 tumors.

The relationship between Bcl-2 overexpression and outcome is shown in Table 3. Six (86%) of the 7 patients who had a poor outcome were Bcl-2–positive, whereas only 6 (23%) of the 26 patients with a good outcome were Bcl-2–positive. Of the 12 patients with Bcl-2 overexpression, only 6 (50%) had a good outcome, while 20 (95%) of the 21 Bcl-2–negative patients had good outcomes. The relationship between poor outcome and overexpression of Bcl-2 in tumor cells was statistically significant ($p = .0047$ by Fisher's exact test). However, there was no significant association between outcome and the proportion of tumor cells staining positive for Bcl-2 (score of 1+, 2+, 3+, or 4+). Table 3 also shows the outcomes distributed according to tumor site and Bcl-2 status. All 14 true vocal cord lesions that were negative for Bcl-2 had a good outcome. For tumors overexpressing Bcl-2, there was no significant difference in recurrence rate between those undergoing surgery versus those undergoing radiotherapy as the primary mode of treatment.

The survival of patients according to Bcl-2 status is shown in Fig 2. The difference in survival between the two groups is statistically significant ($p = .0379$ by log-rank statistic), but the deaths include deaths from other causes. Patients whose tumors did not overexpress Bcl-2 had a 5-year survival of 94.4%, versus 64.8% for patients whose tumors were Bcl-2–positive.

DISCUSSION

In this study, poor treatment outcome and shorter overall survival were significantly associated with Bcl-2 overexpression by the tumor, irrespective of the treatment modality used. These results strongly suggest that Bcl-2 overexpression confers a significant risk of unfavorable treatment outcome in patients with localized SCCHN.

These observations are generally consistent with a recent report by Gallo and Bianchi,[16] who found that Bcl-2 overexpression was a highly significant predictor of a short disease-free interval and decreased overall survival in early-stage SCCHN. In their series, 70% of tumors were in the larynx, and all were treated by irradiation. Our population was different in that most tumors were treated with surgery. The finding that Bcl-2 overexpression has adverse prognostic significance irrespective of the mode of therapy suggests that this subset of patients probably needs more aggressive treatment and closer follow-up. The mechanism proposed by Gallo and Bianchi to account for Bcl-2's prognostic significance, namely, Bcl-2–induced radioresistance, does not apply to our patients who underwent surgery. Although the two studies tracked clinical outcome differently, the re-

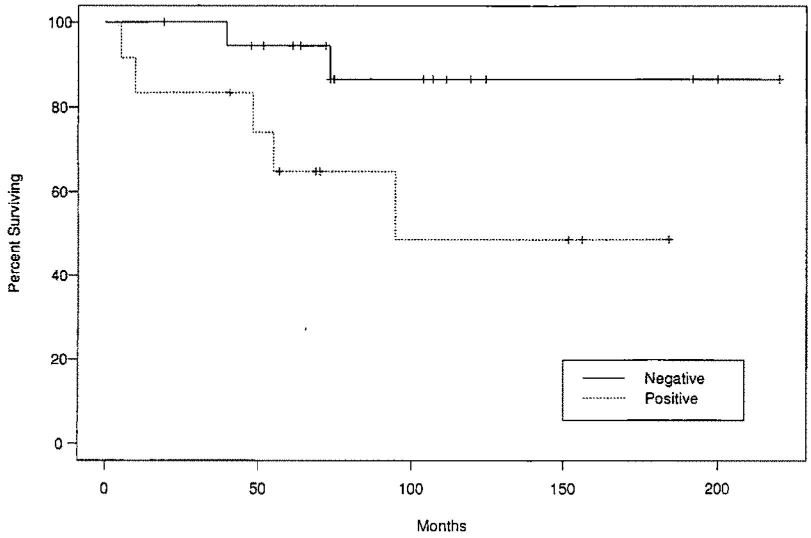

Fig 2. Survival of 33 patients with localized squamous cell carcinoma of head and neck according to Bcl-2 status. Log-rank statistic = 4.3; $df = 1$; $p = .0379$.

sults of both clearly indicate that Bcl-2 overexpression is an important determinant of clinical outcome. Our finding of 36% of relatively early SCCHNs staining positive for Bcl-2 is a higher rate than the 18.4% found by Gallo and Bianchi, but is comparable with the 27% positivity rate found by Pezzella et al[17] in a study of squamous cell carcinoma of the lung.

The mechanisms by which disordered Bcl-2 expression could lead to treatment resistance and shorter survival are speculative at present, but experimental data suggest at least two possibilities. One is that Bcl-2 overexpression prevents spontaneous (not treatment-related) apoptosis in SCCHN, leading to more rapid accumulation of tumor cells for a given proliferation rate, regardless of the type of treatment chosen. Spontaneous apoptosis is known to be an important factor in tumor volume doubling time.[18] Another possibility is that Bcl-2 confers resistance to therapy by blocking treatment-related apoptosis, regardless of the type of therapy used.[4]

Another important problem is that the prognostic significance of Bcl-2 overexpression has been found to vary among different tumors. For example, in the study by Pezzella et al[17] on squamous cell carcinoma of the lung, patients with tumors overexpressing Bcl-2 had a better survival rate than those not expressing Bcl-2. A similar observation has been reported for breast cancer.[19,20] A seemingly trivial explanation for this variability that cannot be overlooked is that different investigators are using different methods for assessing Bcl-2 overexpression and that their data are not comparable. Considering the relative simplicity and robustness of the methods used, however, a more likely explanation is that Bcl-2 expression must be considered in the context of the expression of several other, closely related gene products, particularly Bax, Bcl-XL, Bcl-XS, and Bad.[4] For example, Bcl-2 is thought to function largely by antagonizing the cell death–inducing effect of Bax. If Bax expression is somehow impaired, a cancer cell might be resistant to apoptosis even in the presence of very low expression of Bcl-2, and abnormalities of Bax expression have been documented in human breast cancer.[21] This argument also suggests that assessing the family of related genes instead of Bcl-2 alone in patients with SCCHN may allow more accurate detection of potential poor responders to standard treatment. The variation in the significance of Bcl-2 overexpression alone for different tumor types may also be due to differences in the prevalence of abnormalities of these related genes. Additional work will clearly be needed to sort out this complex system in various forms of human cancer, including SCCHN.

The overall percentage of good outcomes in this study was 79%. This includes patients who remained free of disease after treatment and developed no second primaries during the observation period. These results are consistent with published reports of the response to treatment for these lesions. Only half of the patients with Bcl-2 overexpression had a good outcome, whereas 95% of those who were Bcl-2–negative had a good outcome. Similarly, 5-year survival rates were 94.4% in Bcl-2–negative patients, versus 64.8% in Bcl-2–positive patients. One of the goals of gene marker studies is to identify in advance those patients in whom standard treatment is likely to fail. Our data indicate that Bcl-2 testing may be quite sensitive for this purpose; however, it is not very specific, since half of the patients with Bcl-2–expressing tumors did well. Nevertheless, the presence of Bcl-2 overexpression is indicative of a lowering of the chance for good outcome to 50% in those patients with early SCCHN. If these results are borne out by studies with larger sample sizes, Bcl-2 and other tumor markers may become useful as diagnostic or prognostic tools that will influence treatment of these tumors.

CONCLUSION

The overexpression of Bcl-2 in early lesions in this study predicted a cure rate of 50%, as opposed to the generally expected 90%, suggesting that Bcl-2 is a significant prognostic indicator in early SCCHN. Further studies should be conducted to determine if altering the treatment protocol will improve survival in these patients.

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METHOD AND CLINICAL RESULTS OF A NEW TRANSTHYROTOMY CLOSURE OF THE SUPRAGLOTTIC LARYNX FOR THE TREATMENT OF INTRACTABLE ASPIRATION

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A new procedure has been developed to surgically separate the pharynx from the trachea that employs the best features of the Montgomery technique, but restricts the closure to only the epiglottis and the aryepiglottic folds. The petiole of the epiglottis is plicated to the false vocal folds and the interarytenoid mucosa. It is performed entirely through a midline thyrotomy approach and avoids injury to any of the structures within the rima glottidis. It has been successfully performed on seven very ill patients to date. The surgical decision-making process involved, a complete description of the surgical procedure, and a summary of the patients' preoperative condition, workup, and outcomes are presented and discussed.

KEY WORDS — adult, aspiration, dysphagia, medical ethics, patient report, pediatrics, pneumonia, repair, surgical procedure, thyrotomy.

INTRODUCTION

There are a myriad of disease states that have as a common feature laryngeal incontinence and aspiration. They occur in all ages, from newborns to the elderly, and can range in severity from very minor impediments to life-threatening aspiration. The most common approach to the surgical treatment of aspiration is a tracheostomy and a gastrostomy tube.[1-3] While these interventions might seem effective, they rarely address the underlying disability and can produce significant secondary impairments.[1-4]

There are several procedures that have been designed to address laryngeal incontinence, some of which attempt to preserve natural voice. These interventions range from the radical treatment of narrow-field laryngectomy to a variety of glottic and supraglottic closures and the Lindeman laryngotracheal diversion-separation techniques.[5-8] The reader is directed to Dr Andrew Blitzer's thorough review of these procedures from 1987.[1]

The ideal procedure should be minimally morbid, relatively quick, and very reliable. Furthermore, it should 1) avoid the rima glottidis, thus reducing the risk of a difficult reversal with the attendant sacrifice of voice quality or commissure webs; 2) avoid the track of the recurrent laryngeal nerves to avoid iatrogenic true vocal fold (TVF) paralysis; and 3) restore as normal a swallowing mechanism as possible. This report is of such a procedure. The patient experience accumulated thus far supports the benefit of these considerations in a series of seven patients.

PREOPERATIVE CONCERNS

Patient Selection. Most patients who have a diagnosis of mild aspiration require little to no intervention for their dysphagia beyond counseling, periodic follow-up, and some modification of their diet or swallowing method. Even when aspiration is more serious, it is often self-limited and "aspiration precautions" will still suffice until the patient recovers. Timing is an important concern when considering intervention of this type. Many patients will aspirate for a few days to several weeks after extubation, particularly if they were intubated for a prolonged period or are elderly and/or very ill. Only a small percentage of these patients will begin having infectious or respiratory complications of their chronic aspiration. If a patient is actively infected, a decision must be made weighing the increased risks of general anesthesia with active pulmonary infection against the risk of waiting for the lungs to clear. Issues associated with the loss of voice will be elaborated in the discussion below.

Swallowing, Radiographic, Electromyographic, and Endoscopic Studies. A formal videofluoroscopic study by a qualified speech and language pathologist is an important part of the preoperative workup. The nature of the swallowing deficit should be determined, particularly if there are any modifications of the swallowing technique that may render oral intake safe. The complete study should rule out dysfunction

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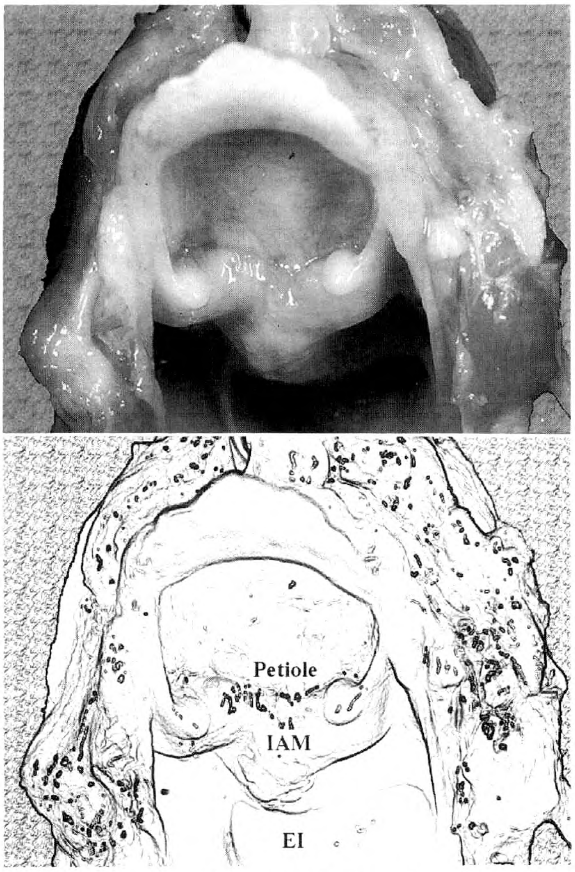

Fig 1. Petiole supraglottopexy. Cadaver dissection depicting finished supraglottic closure from above. Advanced petiole flap is attached to posterior supraglottis as superior layer of closure. Inferior layer of closure deep to this is made up of left and right false vocal folds (FVFs) and is oriented in anterior-posterior direction. Two closure layers are therefore orthogonal to each other. IAM — location of interarytenoid mucosa, EI — esophageal inlet.

of the cricopharyngeus, esophageal dysmotility, intrinsic and extrinsic masses, and gastroesophageal reflux disease. The implications of these findings are self-evident.

Fiber-optic evaluation of swallowing and laryngeal function performed by an otolaryngologist is also necessary to assess other correctable causes of aspiration such as severe laryngeal insufficiency secondary to a widely displaced and paralyzed TVF. The discovery of unilateral supraglottic anesthesia, for example, would explain the basis of the aspiration and should be followed by a workup of the neural deficit, including electromyographic evaluation of the cricothyroid and thyroarytenoid muscle groups.

SURGICAL TECHNIQUE

Overview (Fig 1). The primary goal is to separate the trachea from the pharynx by using the petiole of the epiglottis and the false vocal folds (FVFs) to generate an orthogonal two-layer closure. This is achieved through a midline thyrotomy approach that is extended superiorly, detaching the epiglottis from the thyroepiglottic and hyoepiglottic ligaments. This releases the petiole, allowing it to be advanced posteriorly to reach the interarytenoid mucosa. Horizontal incisions made through the FVFs and dissected into the aryepiglottic folds are then sutured to the advanced epiglottic flap, forming the superior layer of the closure. The petiole is anchored to the interarytenoid space within a muscle and mucosal pocket that is continuous with the horizontal incision of the FVFs just mentioned. The superior closure line is completed with a running suture on both sides. The inferior edges of the FVF flaps are then attached to each other, also with a running suture completing the closure. This layer is oriented in the anterior-posterior direction and is therefore orthogonal to the superior closure line that is largely in the coronal plane.

Surgically Matured Tracheostomy. This technique is described elsewhere^9 and will not be presented in any detail here. The only caveat relating to this procedure is to avoid making the dissection too cephalad. Preferably, the approach to the trachea can be below the level of the thyroid isthmus. This will enable the sealing off of the laryngeal operative field and make the use of suction drains possible.

Midline Thyrotomy (Fig 2). The techniques of midline thyrotomy access are commonly known and will not be described in detail here except as relates to this operation. First, the inferior incision should be taken to or even through the cricoid cartilage. Second, the division of the membranous anterior commissure should be a separate step from the cartilage incision, even if the cartilage is not particularly ossified. Sharp tenotomy scissors or a No. 15 blade can be used to make a precise anterior commissure division once the framework has been divided. Third, the superior end of the anterior mucosal incision is the fibroelastic cartilage of the petiole of the epiglottis. Once this is reached, just 2 to 3 mm above the anterior commissure, the remainder of the anterior dissection is in the deep space between the epiglottis and the thyrohyoid membrane. Laterally, the dissection is within the aryepiglottic folds medial to the pyriform sinus.

Release of Epiglottis (Figs 2 and 3). The epiglottis must be mobilized posteriorly in the preepiglottic space by the lysis of its fibrous attachments, the thyroepiglottic and hyoepiglottic ligaments (Fig 3). When this part of the dissection is complete, the surgeon should be able to palpate a broad pocket anterior to the epiglottis up to the level of the vallecula. The important points of this dissection are as follows. First, once the thyroepiglottic ligament is

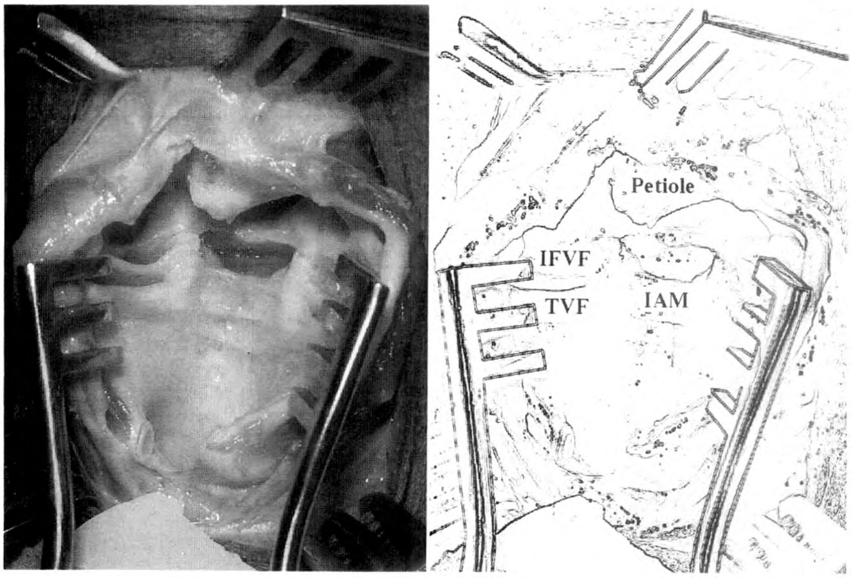

Fig 2. Midline thyrotomy. Midline thyrotomy is widely extended. Specimen has all of mucosal incisions completed, including inferior false vocal fold (IFVF) edge of incised false folds and interarytenoid mucosa (IAM) cuts. Petiole is partially released. Thyrohyoid membrane is divided. TVF — true vocal fold.

lysed, the thyrohyoid membrane should be identified and divided in the midline to the level of the hyoid bone. A self-retaining hinged retractor such as a Weitlaner should be placed high in this area for improved visibility of the upper portion of this dissection. The thyrohyoid membrane will be reconstituted at the end of the closure. The patient can now be placed in the Trendelenburg position. Second, the plane just deep to the thyrohyoid membrane is the dissection plane for approaching the hyoepiglottic ligament; once identified, it should be broadly lysed. The remainder of the dissection is blunt and easily performed.

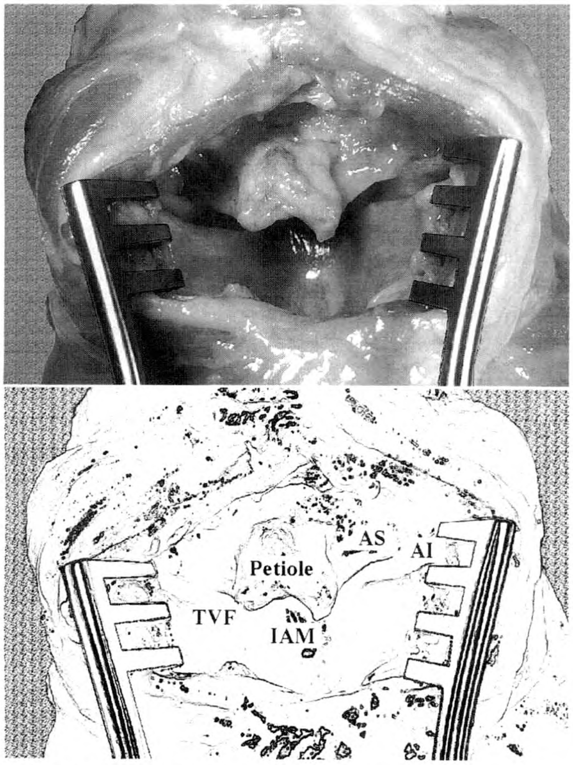

Fig 3. Preepiglottic space dissection. Close-up of released petiole of epiglottis with superior FVF flaps incised and dissected posteriorly prior to anchoring to IAM incision (not visible in this orientation). AS — anterior-superior part of FVF, incised and reflected posteriorly with petiole; AI — anterior-inferior part of FVF that will form inferior layer when it is sutured to same tissue on right side.

Extending Preepiglottic Space Dissection Laterally (Fig 3). The details of this dissection are as follows. First, the preepiglottic space is now dissected laterally toward the superior laryngeal neurovascular pedicle. Care should be taken not to disrupt these structures, since the already disordered swallowing mechanism would only be worsened with such an insult. Second, the above-described pocket is now much wider and extends into the deep space bounded by the aryepiglottic fold medially and the medial wall of the pyriform sinus laterally. The posterior incision can now be made by extending the anterior FVF incision into the pocket just created. The plane of the incision is axial and will go all the way to the arytenoids at this stage.

Incising False Vocal Fold and Interarytenoid Mucosa (Fig 4). The dissection to this point is intended to release the epiglottis. The next moves will generate a tube-like flap of tissue made up of the inferior epiglottis, the superior FVF-aryepiglottic fold, and the interarytenoid mucosa (Figs 2-4). These structures will be collapsed onto each other, with the petiole being advanced to the interarytenoid mucosa and the remaining edges of the incised mucosa being sewn together in an everting manner to complete the superior layer of the closure. The self-retaining retractor is progressively extended as each dissection point is completed and the width of the midline thyrotomy aperture can be increased. The ease with which the anchoring suture can be placed to attach the

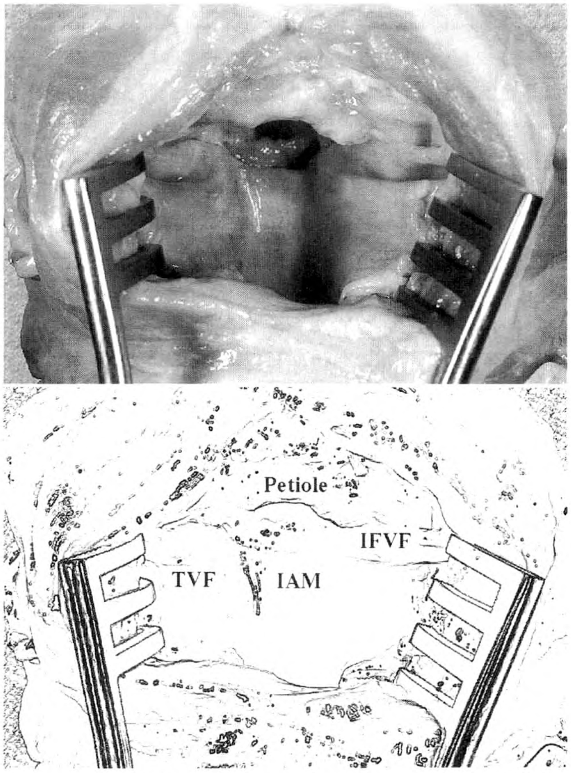

Fig 4. Preepiglottic space dissection and mucosal incisions. Petiole of epiglottis is retracted superiorly to reveal continuous incision that extends posteriorly along posterior FVF, arytenoids, and IAM. IFVF — inferior false vocal fold tissue flap that makes up inferior closure layer.

petiole to the interarytenoid mucosa depends on a wide access to the posterior larynx. The steps are as follows. First, the FVFs are incised anteriorly in the same horizontal plane as the inferior edge of the petiole. This incision should be taken posteriorly to the arytenoid bilaterally. The FVFs should be dissected superiorly until the plane joins the preepiglottic space pocket, forming one large contiguous space to the approximate midpoint of the aryepiglottic fold in the coronal plane. The dissection can easily be taken more posteriorly than this, but there is no advantage, since the anterior tissues will attach to the posterior in a kind of "fish mouth" configuration. Second, the posterior mucosal incisions beyond the dissection described above are raised into flaps large enough to allow for the anchoring of the running suture line that will attach them to the anterior tissues. (About 2 to 3 mm is sufficient.) This posterior incision is now extended over the arytenoid towers and onto the interarytenoid mucosa. The mucosa is very adherent to the arytenoid cartilage, and a rim of tissue to which to suture may be difficult to generate. If so, the cartilage is soft and the suture can actually be anchored to the arytenoid itself. Still, there must be a denuded zone so that the mucosa does not fuse back together and generate a fistula rather than heal to the advanced flap. Alternatively, the arytenoid cartilage can be incised. This will not affect the motion of the arytenoid. Third, the interarytenoid mucosa incision is very important, since it is the anchor point of the superior and inferior closure layers. The incision must be high enough on the rim of this tissue to avoid the posterior commissure, and deep enough to include some of the interarytenoid muscle with the upper and lower attachments.

Attaching Petiole and Completing Superior Closure Layer (Figs 5 and 6). The upper closure layer is completed as follows. First, the petiole is tagged at two points with the 4-0 monofilament, long-lasting absorbable suture on an RB-1 needle. This stitch must be imbedded into the fibroelastic cartilage of the petiole. This tissue is very dense and resists the passage of the taper needle. Each stitch is then attached to the muscle and submucosa of the interary-

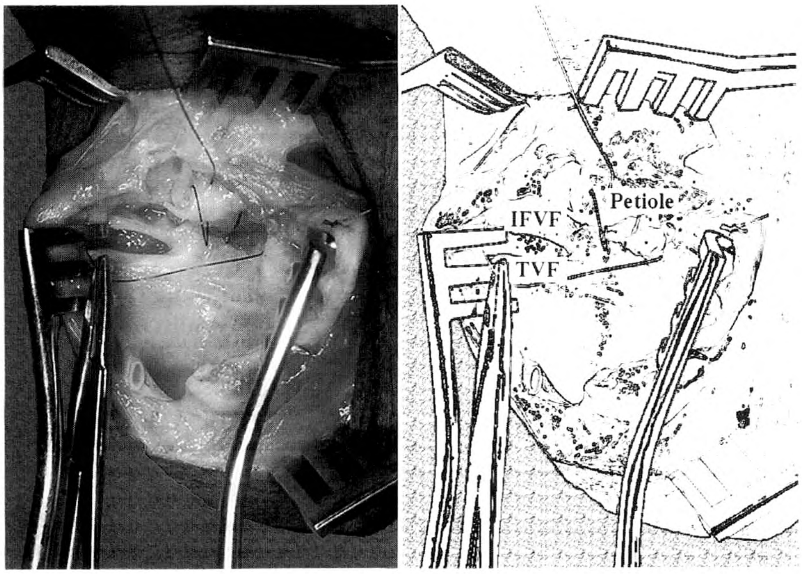

Fig 5. Anchor of petiole. Petiole is anchored by two sutures into pocket of muscle and mucosa made in interarytenoid area. Each of these sutures is then used to run closure laterally to seal off pharynx from trachea as superior layer.

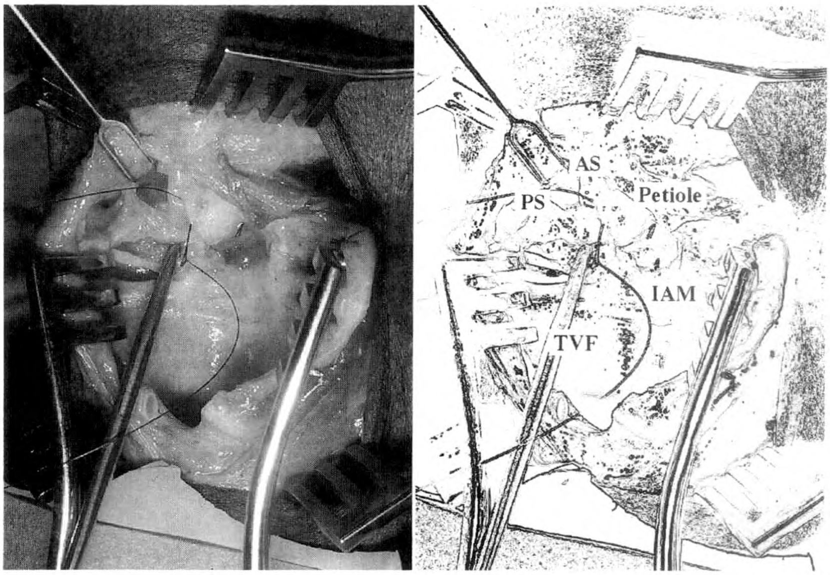

Fig 6. Superior closure layer. Suture used to anchor petiole is shown being attached to posterior-superior FVF flap (PS) in preparation for running closure of superior layer. Tissue hooks are exposing pharyngeal side of closure and lifting anterior-superior FVF (AS). These two edges are being fused to form superior closure. IAM — IAM inferior flap.

tenoid mucosa superior flap. The petiole is "dunked" into this pocket, and the sutures are tied down. Each anchoring suture is then run laterally in a manner to evert mucosa toward the pharyngeal lumen. The sutures are tied to themselves at the completion of this suture line. Identifying the mucosal edge in this part of the closure is made much simpler if a single fine tissue hook is held at the apex of the aperture to be closed.

Inferior Closure Layer (Figs 7 and 8). Attention is turned back to the petiole that is attached superficially to the upper layer of the interarytenoid mucosa flap. Ultimately, the inferior part of the interarytenoid mucosa flap will cup the deep space of the petiole and serve as the anchor of the inferior closure line. The self-retaining retractor needs to be progressively relaxed during this part of the closure. At the end of the procedure, the aperture will be about as narrow as it was at the start of the midline thyrotomy, prior to releasing the epiglottis. The steps are as follows. First, the remaining inferior flap of tissue is examined to make sure there has been enough of an elevation to make attaching the left and right sides simple and tension-free. If not, the fibrofatty tissues of the FVFs should be elevated on either side. A Freer elevator can be useful to elevate the mucosa off the arytenoid body cartilage in preparation for the inferior layer of closure. Second, the self-retaining retractor should be released one or two notches. Third, another suture is set into the deep surface of the petiole fibroelastic cartilage. Using a "tendon" double-pass technique to set this suture will ensure a durable anchor. Then, this suture is attached to the inferior interarytenoid mucosa muscle and submucosa and secured with a tie. These needle placements are more difficult than those of the upper layer, since the aperture is collapsing. It is important to avoid trapping

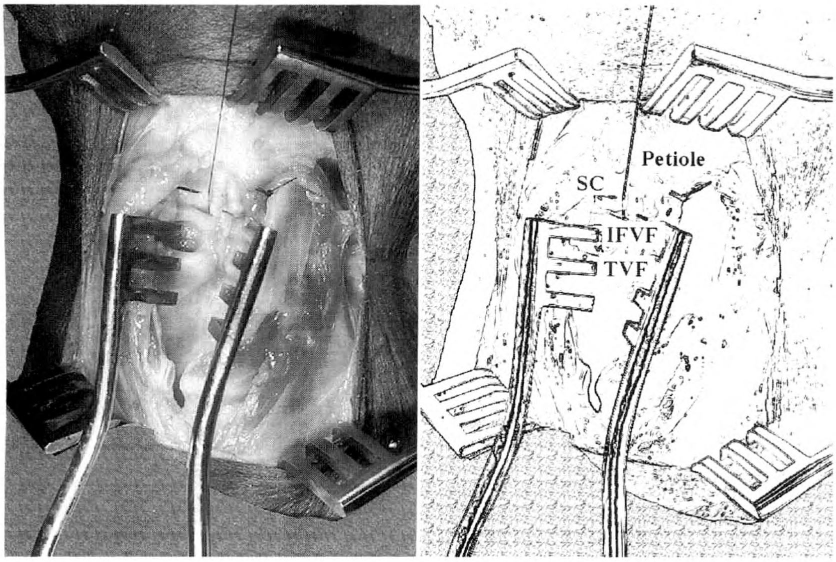

Fig 7. IFVF closure. Superior closure (SC) is complete. IFVF flaps are now being sutured together in midline as Weitlaner retractor is being progressively relaxed.

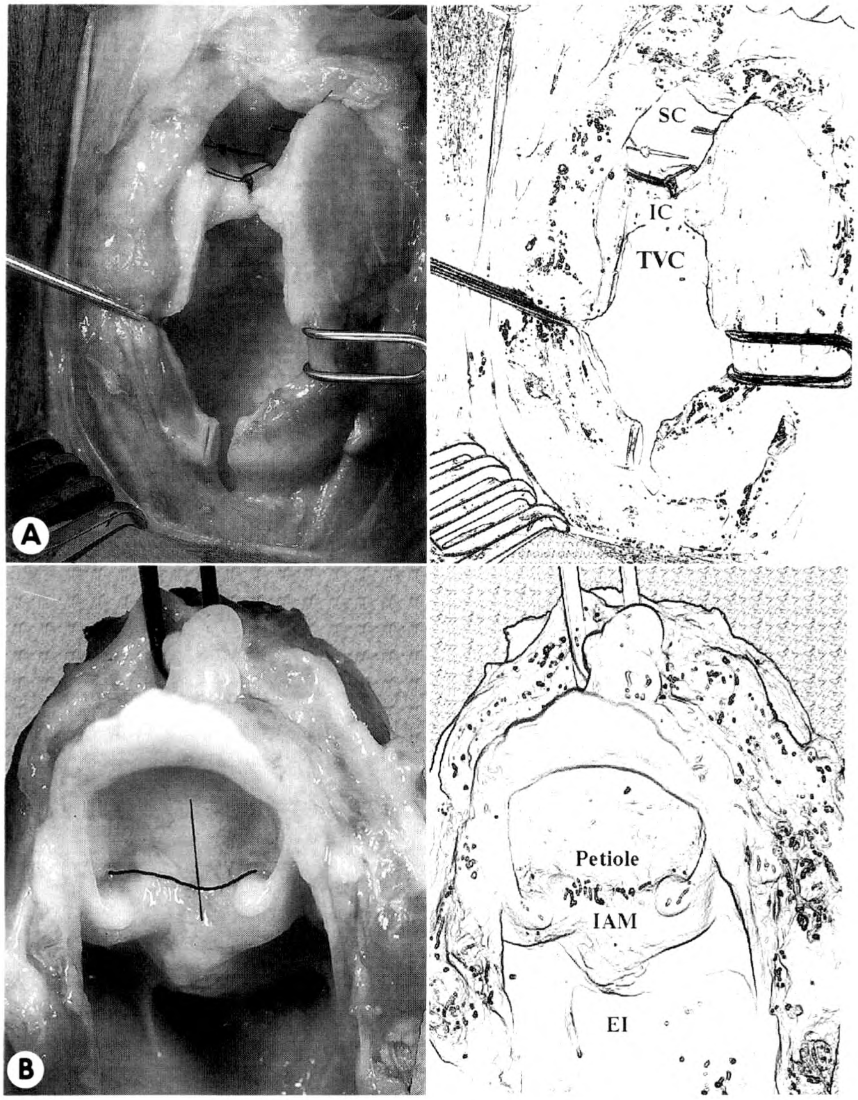

Fig 8. Completed closure. A) Anterior view. Superior closure (SC) and inferior closure (IC) layers are now complete. In actual closure operations, inferior layer is attached to preepiglottic soft tissues so as to obliterate this dead space. Here, separation serves to highlight two distinct layers. B) Superior view. This view illustrates closure configuration of two closure layers, with superimposed lines depicting their orientation. Vertical line depicts IC layer in A, and horizontal line is tracing of SC layer. IAM — location of interarytenoid mucosa, EI — esophageal inlet.

mucosa, since these tissues are replete with mucous glands and could potentially form mucoceles. If the mucosa is joined directly from one side of the FVFs to the other, a pocket is created that might become filled with blood or mucus and get infected. The dead space between the two layers can be progressively obliterated as the FVFs are approximated by incorporating the preepiglottic fibrofatty tissues with each completed pass, ie, suture to left FVF, then to right FVF, and then through the preepiglottic fibrofatty tissues, and so on. This suture is tied to itself anteriorly, and the self-retaining retractor is removed. Figure 8A depicts the finished closure with a space between the upper and lower closure layers so as to render them distinct for purposes of illustration only.

Closing Midline Thyrotomy. The method of closing the midline thyrotomy is routine. Two suggestions are 1) to insert a suction drain into the deep space of the closure to drain fluid that may accumulate and 2) to reapproximate the anterior commissure carefully so as to prevent webbing. The latter consideration is particularly important in patients who may eventually have a reversal procedure.

Postoperative Care. Although the stoma has been surgically matured, intensive care unit monitoring is recommended after this type of case. Apart from the standard risks of tracheostomy, there is still a small risk that the patient's cannula may become displaced and the fresh tract disrupted. Therefore, it may be appropriate for the surgeon to review with the intensive care unit staff and intensivist the change in the patient's anatomy that has taken place, specifically, that there is no longer the option to intubate from above if an airway emergency is encountered. Furthermore, nasogastric tube manipulation or replacement should be performed with direct visualization by the surgeon or an experienced endoscopist to avoid disruption of the closure. Drain outputs have been very modest even in patients that had to be returned to intravenous heparin. Patients returning to

TABLE 1. PATIENT SERIES

	Patient						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Age at time of surgery	20 mo	10 y	62 y	67 y	44 y	65 y	70 y
Sex	M	F	M	M	M	M	M
Primary diagnosis*	Herpes encephalitis	MR/CP	Strokes	Strokes	Laryngeal paralysis	Strokes	Pontine stroke
Secondary diagnosis	MR/CP	Seizures	Diabetes	HTN	Diabetes	HTN/DM	HTN/DM
PO status							
Preoperative	NPO	NPO	NPO	NPO	NPO	NPO	NPO
Postoperative	Partial PO	Subtotal PO	PO	PO with sup.	PO	PO with sup.	GT; PO rehab§
Cognitive status	Encephalopathic	Severely impaired	Intact	Mildly impaired	Intact	Severely impaired	Locked in
Voice/speech preoperatively	Faint cry, no reactive sounds	Grunting, no speech, minimal reactive sounds	Grunting, severe dysarthria	Grunting, severe dysarthria	Very breathy, otherwise intact	Grunting, severe dysarthria	Few grunts, no speech
Current mode of communication	Nonverbal	Nonverbal	Gestures and facial expression	Text communicator	Electrolarynx	Head and eye motion	Head and eye motion
Residence							
Preoperative	Home	Home	Rehab facility	Home	Inpatient	Nursing home	Inpatient
Postoperative	Home	Home	Home	Home	Home	Nursing home	Pending
Tracheostomy (preoperative)	No	Yes	No	Yes	Yes	No	No
Gastrostomy							
Preoperative	Yes	Yes	Yes	No	No	Yes	No
Postoperative	Yes	Yes for sup.	Yes for sup.	Yes for sup.	No	Yes for sup.	Yes
Length of follow-up	15 mo	14 mo	13 mo	12 mo	10 mo	8 mo	2 mo§
Complications	None	Revision, tracheitis†	None	None	None	None	None

MR/CP — mental retardation/cerebral palsy, HTN — hypertension, DM — diabetes mellitus, PO — per os, NPO — nil per os, sup. — supplementation, GT — gastrostomy tube.

*Other than intractable aspiration.

†See text regarding patient 2.

§Lost to direct follow-up at 2 months postoperative.

enteral anticoagulation can have their first dose on the night of surgery and receive intravenous heparin as early as the next morning. No bleeding complications were encountered with this regimen. All of the patients in this series were cleared for oral diets at 1 week postoperatively, except patient 5 (see below).

PATIENT RESULTS

The outcomes of all of the patients in this operative series are summarized in Table 1. The details described below are not intended to be complete histories of their postoperative course, but rather, to highlight some important features of each case that may prove useful to the airway clinician.

Two pediatric and five adult patients have thus far been treated with this method of supraglottic closure. The pediatric patients were 20 months old and 10 years old at the time of surgery. The adults' ages ranged from 44 to 70, with a mean of 61.6. All patients had a neurologic basis for their aspiration disorders. All had sustained infectious and respiratory complications of their aspiration, and six had

TABLE 2. COMPARISON OF SURGICAL CLOSURE TECHNIQUES

	Petiole Supraglottopexy	*Montgomery Glottic Closure*[8,9]	*Lindeman Laryngotracheal Separation*[5]	*Habal and Murray Flap Epiglottoplasty*[7]	*Biller Tubed Epiglottoplasty*[6]
Surgical approach	Thyrotomy	Thyrotomy	Paratracheal dissection	Pharyngotomy	Pharyngotomy
Destruction of true vocal fold epithelium	No	Yes	No	No	No
Risk to RLN	No	No	Yes	No	No
Tracheostomy dependence	Yes	Yes	Yes	Yes	Yes
Forms blind pouch	No	No	Yes	No	No
Preservation of voice	Possible	No	No	Possible	Possible
Reversibility	Possible	Possible	Possible	Possible	Possible

RLN — recurrent laryngeal nerve.

documented recurrent aspiration pneumonia. Out of the four compos mentis adult patients, one had the ability to speak and two were severely dysarthric. One patient was "locked in," with his level of cognition being difficult to assess. The two pediatric patients were considered neurologically devastated.

None of the patients in the series were receiving oral nutrition prior to surgery. All changed to at least partial oral nutrition, except patient 7 (see below). No patient had any further respiratory infections, except patient 2, who had one episode of bacterial tracheitis secondary to topical steroid treatment of tracheostomy granulation tissue. All patients returned home after their closure operations, except patient 6, who did not have family that could care for him, and patient 7, who was at a rehabilitation facility.

No significant wound complications were encountered. The only patient requiring a revision procedure was the 10-year-old (patient 2), who was initially given an abbreviated procedure using only the FVFs to attempt her closure without advancing the petiole of the epiglottis. Her revision operation employed the procedure described here and was successful thereafter. The last patient in the series had a clinically insignificant leakage discovered just prior to his transfer to an outside rehabilitation facility. It resulted in a delay in his return to oral and swallowing rehabilitation. According to his family, he has not had any further pulmonary infections since his closure and appears to have spontaneously closed the leaking area. He is being followed up by the otolaryngology service of another institution, and follow-up data were not available for this publication.

CASE REPORT

Patient 5 was status post a simultaneous kidney-pancreas transplant. He had severe persistent posttransplant hyperemesis and aspiration secondary to gastroparesis and laryngeal paresis, along with peripheral neuropathy presumed to be diminishing his laryngeal sensation. He was also 2 years status post a partial pharyngectomy, a modified radical neck dissection, and postoperative radiotherapy for a T1N1Mx squamous carcinoma of the oropharynx. His serum albumin was 1.8 g/dL, indicating severe malnutrition. He was also being treated for graft rejection with multiple immune suppressants, including 200 mg/d of prednisone. Therefore, there were multiple complicating factors preventing him from protecting his airway from his hyperemesis and giving him a high likelihood of surgical complications.

I initially performed a tracheostomy with the hope that the flow of his gastric contents into his tracheobronchial tree could be slowed. His condition continued to worsen, with an unabated flow of emesis into his lungs. He was taken back to the operating room, and a supraglottic closure was performed. His tissues were typical of postradiotherapy mucosa: friable and edematous. He was given nothing by mouth for a full 14 days postoperatively to give him time to heal. His pulmonary status promptly improved after his laryngeal closure. No postoperative complications occurred. Once he was gradually started on oral nutrition, his general condition rapidly improved, and he was able to be transferred to a rehabilitation facility at 4 weeks postoperative and was discharged to home 3 weeks later. He has had no further respiratory or gastrointestinal problems since then, and is being considered for a reversal of his closure.

DISCUSSION

Comparison of Petiole Supraglottopexy With Other Laryngeal Closure Techniques. There are numerous techniques for restoring laryngeal continence to which the petiole supraglottopexy (PSGP) can be compared. A type of laryngeal closure procedure designed to be performed through a relatively small wound was described by Dr William Montgomery in 1975.[8] Through a midline thyrotomy, the epithelium of the endolarynx is stripped and the tissues of the FVFs and TVFs are plicated to each other in a series of interrupted sutures. The initial version of the procedure was found by some to be prone to breakdown, and a revised version was described by Sasaki et al[10] that included a muscle flap to generate a third layer of closure and reduce the rate of breakdown. There is still a risk of breakdown, and the difficulty of reversing the closure is thought to be greater with the added scar tissue that forms between the vocal folds.[1]

The PSGP retains the advantages of the Montgomery procedure in that it can be performed entirely through a midline thyrotomy approach, is potentially reversible, and does not form any type of blind pouch like the Lindeman method. The PSGP is an improvement on the Montgomery closure, since it does not require the plication of the TVFs and thus does not risk destroying the phonation mechanism. The potential for posterior web formation is low, since the true posterior commissure is not violated. No patient has thus far developed either a posterior or anterior glottic web, according to retrograde endoscopy. Furthermore, the PSGP has thus far been completely reliable without the use of secondary muscle flaps, even when performed on very sick patients. The comparison of the PSGP to the Montgomery procedure and other aspiration surgeries is summarized in Table 2.[5-9]

Outcomes and Quality of Life Issues. A definitive outcomes comparison is not possible with this data set, since all of these patients have been treated surgically. It is clear that definitive intervention subverted the most likely outcome of the disease processes in the cases outlined. The preoperative and postoperative conditions and a list of several quality of life factors are summarized in Table 1. Except for the one mild infection in patient 2, no significant airway infections have occurred in any of these patients postoperatively.

The care needs of the two pediatric patients dropped dramatically. In both cases, the caregivers are now able to feed the children orally. This has opened venues of interaction, reinforcement, and even bonding that were not previously possible. The tradeoff was the loss of whatever communication potential there was in the nonspeech sounds these patients made. Neither were capable of any degree of speech preoperatively, but both were able to cry out if in distress. This loss in both cases was the main concern of the caregivers preoperatively. In both cases they report being very pleased that they decided to proceed with the operation. Patient 1 was treated as an infant and was constantly with his caregiver, obviating the need for this form of communication. Patient 2 was connected to apnea and saturation monitors that assisted her guardian with care and surveillance.

In the five adults, issues of communication were also very important, despite the fact that three of the five were severely dysarthric, to the point of being incapable of intelligible speech. Except for patient 7, who is still very early in his recovery, they are able to use alternate means of communication, such as bells to get help if they need it, and are comfortable with their communication abilities. Patient 5 is using his electrolarynx very well. Patient 6, the most obtunded of the group, was so minimally communicative preoperatively that his laryngeal closure has had little impact on his level of interaction with his environment. He has returned to a near-complete oral diet and appears to be enjoying his recovery of that capacity. Five of the seven patients are at home, and none have had any significant problems. These patients, who would almost certainly have been readmitted to the intensive care unit, have not had a single admission of consequence following their laryngeal closure, as of the time of this writing.

SUMMARY

The PSGP procedure for closing the larynx has been found to be a durable and easily performed method for the definitive surgical treatment of intractable aspiration. This has been demonstrated in both pediatric and adult patients, with many improvements in quality of life and health. The ethical and medicolegal issues are complex. However, one can clearly conclude that the definitive treatment of a disease process like intractable aspiration can subvert a spiral of recurring illness and intensive care treatment. The cost-benefit analysis and the outcomes research remain to be carried out.

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COMMENT

Dr Castellanos has beautifully presented a new technique for laryngeal closure. His description of the technique and accompanying Figures should serve as a model for any descriptions of new procedures in our field, as one could perform the procedure after reading this article. He honestly presents his complications and provides a very nice ethical discussion on the merits of his technique. However, similar to other published reports, no results of reversal are given. Probably, this reflects appropriate patient selection, in that the majority of patients who undergo these procedures do not have reversible disease. Those patients who might have reversible disease (head trauma) can effectively be managed by short-term (months) tracheotomy. Conceptually, I have a hard time understanding why this new technique would not be subject to significant supraglottic stenosis following attempted reversal; supraglottic stenosis has not been as easy to repair, in my experience, as glottic or subglottic stenosis.

Therefore, if I believed that reversal was truly going to be an option, I would prefer to use the Lindeman laryngotracheal separation technique, as I think this is least likely to produce stenosis when reversed. If the procedure is performed between the second and third tracheal rings, no danger to the recurrent laryngeal nerves should occur, if dissection proceeds immediately beneath the pretracheal fascia. Even though concern has been expressed by Dr Castellanos and others regarding the blind pouch that forms as a result of this technique, I personally have never seen any complications of it, nor have I heard of any in the halls of our national meetings.

What is desperately needed is a paper on the outcomes of reversal of all of these techniques. Failing that, all of the surgical procedures described are successful, and I am optimistic that Dr Castellanos' method will also endure, as well. I salute the author on a well-done report.

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DIAGNOSIS AND MANAGEMENT OF LEFT MAIN STEM BRONCHUS COMPRESSION

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There are four major variants of congenital vascular tracheal compression: innominate artery, aberrant subclavian, aorta or aortic arch anomaly, and pulmonary artery sling. These forms of vascular compression typically involve the trachea and/or the right main stem bronchus. We present eight cases of congenital vascular compression involving the left main stem bronchus. These cases represent a poorly understood variant of vascular tracheal compression. This variant represents approximately 10% of our pediatric tracheobronchial compression or stenosis patients. The finding, both noted endoscopically and now illustrated by magnetic resonance imaging, is caused by compression of the left main stem bronchus between the descending aorta and a portion of the pulmonary artery. Frequently, the descending aorta is in an abnormal anterior position with relation to the thoracic spine. Recognition of this entity is important in our experience and has influenced clinical management. In four of eight children, it required a surgical procedure directed toward the relief of the left main stem compression.

KEY WORDS — aorta, bronchus, congenital disorders, trachea, vascular compression.

INTRODUCTION

The workup of infants and children with respiratory symptoms typically consists of high-kilovoltage x-ray examination, fluoroscopy, barium swallow study, and endoscopy. Endoscopy is done carefully, with full attention paid to both the structure and function of the laryngotracheal complex, as well as the tracheobronchial tree. Occasionally, one can find compression of the tracheobronchial tree by a mediastinal structure. This may appear endoscopically as some variation of compression of the distal trachea and/or the main stem bronchus. Classically, it is caused by either the innominate artery (compression of the anterior wall of the distal trachea), aberrant subclavian artery (compression just above the right main stem bronchus), aortic arch anomaly (tracheal compression), pulmonary artery sling (involvement of the trachea and right main stem bronchus), or aorta (left side of main trachea).[1] Often, the presentations of these anomalies have a classic appearance both endoscopically and on x-ray examination. Recently published in the radiologic literature is a report of two cases of left main stem bronchial compression between the descending aorta and main pulmonary artery found on magnetic resonance imaging (MRI) and not previously described by plain film, fluoroscopy, or computed tomography (CT). The article, however, was without clinical or endoscopic description.[2] Previous to this report, we, as well as others, had noted the finding endoscopically, but could not explain it anatomically on the basis of present-day imaging. In 1993, we had moved toward the use of MRI in the workup of these lesions and also noted the same findings as the previous authors. Furthermore, this finding was consistent with our endoscopic examination.

We now have experience in eight children with compression of the left main stem bronchus between the descending aorta and the main, right, or left pulmonary artery. Abnormal vascular anatomy and segmental bronchomalacia, probably secondary to compression, appears to be the pathogenesis of this newly recognized phenomenon. Treatment varied depending on the severity of the lesion. The patients in this report presented between January 1993 and December 1995 and are currently being followed up.

CASE REPORTS

Case 1. This patient presented term at birth with respiratory distress and cyanosis requiring intubation. Echocardiography revealed tetralogy of Fallot with pulmonic stenosis and insufficiency, as well as enlargement of the main and left pulmonary arteries. The patient underwent division of a patent ductus arteriosus and placement of a transannular autogenous pericardial pulmonary outflow patch. He failed

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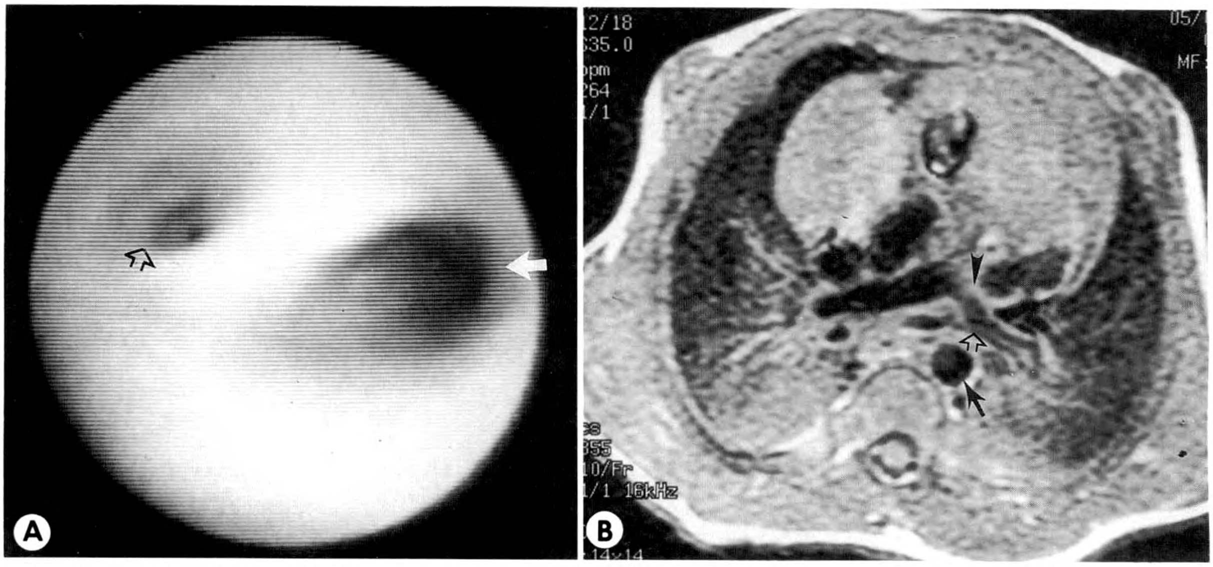

Fig 1. (Case 3) A) Endoscopic photograph, showing that left main stem bronchus is narrowed (open black arrow). Right main stem bronchus (solid white arrow) has normal caliber. B) Nonenhanced, T1-weighted axial magnetic resonance image of same patient, showing that approximately 75% of luminal diameter of thoracic aorta (solid arrow) lies anterior to adjacent vertebral bodies. Left main stem bronchus (open arrow) is moderately narrowed as it crosses between aorta and pulmonary artery (arrowhead).

extubation, so bronchoscopy was performed, which revealed a pulsatile compression of the carina and left main stem bronchus. Then, MRI revealed that the thoracic aorta was located directly anterior to the vertebral column instead of in its normal position along the left side. The left main stem was diffusely narrowed and appeared to be compressed between the anomalous thoracic aorta and the right pulmonary artery. At age 3 months, the patient underwent closure of his ventriculoseptal defect and reconstruction of the pulmonary artery outflow tract, with reduction in size of the main and right pulmonary arteries to normal diameter. Postoperative bronchoscopy, however, revealed continued narrowing of the left main stem bronchus despite the attempts to reduce the pulmonary artery size. It should be noted that intraoperative bronchoscopy was not utilized by the cardiac surgeon in this case. The patient failed extubation because of continued positive pressure requirements and was ultimately discharged home with a tracheotomy and continuous ventilator assistance. He is currently stable, now over 1 year of age, and ventilator-dependent.

Case 2. This patient presented with cyanosis at birth, requiring ventilatory support. An echocardiogram revealed tetralogy of Fallot with absent pulmonary valve leaflets, as well as massively enlarged pulmonary arteries. The medical workup also revealed DiGeorge's syndrome. The patient underwent closure of his ventriculoseptal defect, allograft enlargement of the pulmonary outflow tract, and reduction left pulmonary arterioplasty in an attempt to reduce left main stem compression. He failed to wean off the ventilator postoperatively. It should be noted that intraoperative bronchoscopy was not used in this procedure. Flexible bronchoscopy performed 1 week later was consistent with bronchomalacia and extrinsic compression. Both main stem bronchi were narrowed, with the left narrowing far greater than the right. An MRI scan revealed aneurysmal dilation of the right and left pulmonary arteries, with compression of both main stem bronchi by the pulmonary arteries. The compression on the left side was far more severe, with partial obliteration of the bronchus between the dilated left pulmonary artery and the descending aorta. The aorta was relatively anterior with respect to the vertebrae. In addition, the patient had an aberrant right subclavian artery that passed posterior to the esophagus.

The patient's cardiac and pulmonary status continued to decline. He was considered to be an unsuitable candidate for tracheobronchial stenting, given the nature of his airway disease and the presence of significant cardiac and immune disease. Ventilator support was withdrawn, and the patient died at age $3^1/2$ months.

Case 3. This patient presented at 3 weeks of age with intermittent stridor and increased work of breathing. She was noted on endoscopy to have laryngomalacia and minimal subglottic stenosis. The patient was discharged, but developed respiratory difficulty 1 month later. Repeat endoscopy revealed worsening of the subglottic stenosis, unchanged laryngomalacia, and 20% compression of the left main stem bronchus (Fig 1A). A tracheotomy was performed to sort out

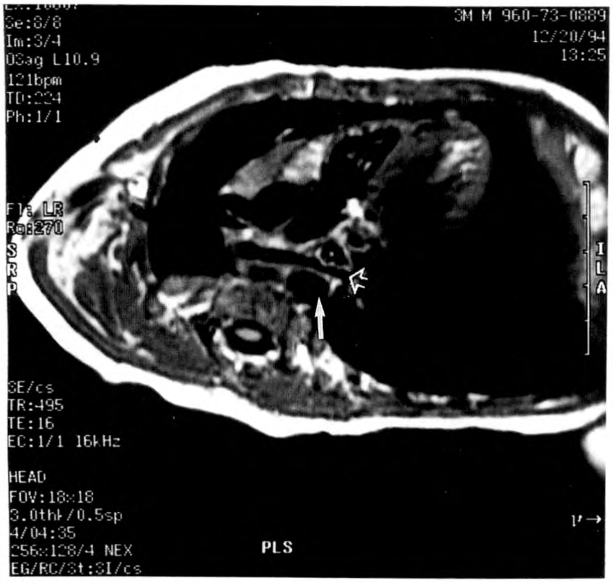

Fig 2. (Case 5) Nonenhanced, T1-weighted magnetic resonance image taken in oblique-sagittal plane through long axis of left main stem bronchus (open arrow). Note focal compression of left main stem bronchus as it crosses between thoracic aorta (solid arrow) and main pulmonary artery. Also note normal diameter of surrounding bronchial segments. Cuts through long axis of bronchus showed focality of compression well. Axial plane afforded better visualization of relationship of aorta to vertebrae.

whether respiratory difficulty was due to proximal or distal compression or both. An MRI scan showed mild diffuse narrowing of the left main stem bronchus with mild compression between an anteriorly malpositioned descending aorta and the left pulmonary artery (Fig 1B). The child did well with a tracheotomy, so at age 2 months, with a body weight of 3 kg, the patient underwent a supraglottoplasty and anterior graft laryngotracheoplasty with auricular cartilage, but failed extubation. The child is stable at over 1 year of age with a tracheotomy. She has gained weight fairly well and is being evaluated for repeat laryngotracheoplasty.

Case 4. This patient presented at age 5 weeks with respiratory distress and was intubated at a local hospital, where he also underwent bronchoscopy revealing severe tracheal narrowing. The child was transferred and underwent MRI that revealed a sling-ring complex (aberrant left pulmonary artery with complete tracheal rings). The complete rings formed a funnel-shaped trachea (Cantrell-Guild type II). In addition, there was an anomalous airway branching pattern consistent with bridging bronchial anatomy. (The right upper lobe bronchus branched off the trachea at the level at which the carina would normally be, and an intermediate bronchus continued from that branch point to the level of the left atrium, at which point this bronchus bifurcated into the right and left main stem bronchi.) The patient underwent transplantation of the left pulmonary artery onto the main pulmonary artery and resection of the complete tracheal rings with primary reanastomosis and creation of a trifurcating neocarina with intraoperative endoscopy. At age 8 months, repeat endoscopy revealed moderate midtracheal narrowing and approximately 80% pulsatile compression of the left main stem bronchus. An MRI scan showed mild focal narrowing of the upper trachea at the level of the crossing innominate artery (not noted on the original MRI), and marked narrowing of the middle portion of the left main stem bronchus between the left pulmonary artery and an anteriorly displaced descending aorta. Repeat endoscopy 1 year later revealed improvement of the midtracheal and left main stem bronchial narrowing. The patient is now $2^1/2$ years old with baseline noisy breathing, but thriving, with no retractions.

Case 5. This patient presented as a newborn with cystic fibrosis. High-kilovoltage films and fluoroscopy obtained at $1^1/2$ months of age revealed focal narrowing of the trachea at the thoracic inlet and mild, diffuse narrowing of the distal trachea and main stem bronchi. At age 3 months, he was intubated for 1 week secondary to a bronchopulmonary infection. An MRI scan was obtained and revealed focal narrowing of the main stem bronchus as it passed between the descending thoracic aorta and the left pulmonary artery; the descending aorta was located in a prevertebral position. The patient was eventually weaned from the ventilator, but returned 8 months later with a similar scenario. Bronchoscopy at the time revealed a 50% midtracheal anterior compression and a 70% pulsatile compression of the left main stem bronchus. Repeated MRI showed focal narrowing of the trachea at the level of the innominate artery, as well as the previously noted left main stem bronchus narrowing between the main pulmonary artery and the anteriorly displaced descending aorta (Fig 2). The patient did not require surgical intervention. Of interest, his symptoms improved markedly upon cessation of bronchodilator therapy. The patient is currently $1^1/2$ years old and asymptomatic. Recent bronchoscopy showed a slight improvement in tracheal dynamics.

Case 6. This patient was admitted at 4 months of age with a 1-month history of coughing and wheezing. Chest radiographs were consistent with obstructive emphysema of the left lung. High-kilovoltage airway films were nondiagnostic. Bronchoscopy revealed pulsatile, slitlike narrowing of the left main stem bronchus. An MRI scan revealed a 1.3-cm-long area of narrowing in the mid-left main stem, between a normal right pulmonary artery and an abnormally positioned descending aorta located directly anterior to the vertebral column. An initial trial of conservative therapy failed, and the patient experienced recurrent respiratory exacerbations. At age 6 months, the patient underwent left lateral thoracotomy for possible aortic suspension. A thick, fibrous ligament was noted extending from the base of the ductus arteriosus and wrapping circumferentially around the left main stem bronchus. This unusual ligamentous structure was lysed. Palpation of the underlying bronchial segment revealed severe bronchomalacia. Intraoperative bronchoscopy revealed persistent left main stem narrowing that largely resolved upon pulling the aortic arch posteriorly; thus, the arch was suspended posteriorly from a vertebral body. The patient is currently 2 years old and requires daily bronchodilator therapy for chronic cough and exertional dyspnea.

Case 7. This patient presented at age 7 months with a history of noisy breathing and frequent choking and gagging since birth. She had been found to have gastroesophageal reflux and was being treated medically at presentation. She underwent bronchoscopy that revealed 50% narrowing of the left main stem bronchus. An MRI scan revealed diffuse narrowing of the left main stem bronchus between the main pulmonary artery and an anteriorly located thoracic aorta. Given the patient's mild symptoms, no therapeutic interventions were taken. She remains stable at age 10 months. Her symptoms are essentially unchanged.

Case 8. This patient was intubated in his third week of life for respiratory distress and required positive pressure ventilation. Endoscopy revealed left main stem compression. An MRI scan showed anterior displacement of the descending aorta with compression of the left main stem between the aorta and the main pulmonary artery. Posterior suspension aortopexy, as well as pulmonary arteriopexy, was performed with endoscopic guidance. While suspension sutures were placed, an otolaryngologist verified that luminal narrowing was alleviated. The patient was weaned from the ventilator postoperatively and discharged, and is currently stable.

DISCUSSION

The eight cases presented herein represent an entity of vascular tracheal compression not previously well understood. The pathogenesis of this entity appears to be multifactorial, but is primarily related to the anatomy of the descending aorta and pulmonary artery. The main pulmonary artery originates from the right ventricle, just anterior and to the left of the aortic outflow tract. As the aorta arches superiorly and to the left, the pulmonary trunk dives posteriorly, just inferior to the arch, and bifurcates into the right and left pulmonary arteries. This bifurcation is adjacent to the anterior surface of the proximal left main stem bronchus and/or the carina. The posterior surface of the left main stem, in turn, abuts the anterior surface of the descending aorta. Thus, the left main stem is located between the pulmonary arteries and the descending aorta posteriorly.

Anterior displacement of the aorta or posterior displacement of the pulmonary arteries or dilation of any of these vessels could, therefore, compress the left main stem bronchus. Furthermore, the vessels might, from normal anatomic locations, compress the bronchus if its cartilaginous skeleton was intrinsically weak because of chondromalacia. Some patients may have congenital or primary bronchomalacia, while most probably develop secondary bronchomalacia because of chronic compression of the airway by anomalous vascular structures. Analysis of our cases suggests that all such mechanisms occur, but the principal etiologic factor appears to be anterior displacement of the thoracic aorta.

A relatively anterior course of the descending thoracic aorta appeared to be the most common cause of left main stem bronchus compression in our patients, but it was not the only cause. In all of our patients, at least 50% of the luminal diameter of the aorta was located anterior to the adjacent vertebral bodies on MRI. In three cases, the aorta was completely prevertebral in location (cases 1, 5, and 6). An additional component in two patients was enlargement of the left and/or main pulmonary arteries, which further compressed the left main stem bronchus (cases 1 and 2). Another patient had intrinsic airway abnormalities, including tracheobronchomalacia, that appeared to make the bronchus more susceptible to compression (case 4). In fact, half our patients had findings consistent with tracheomalacia and/or bronchomalacia (cases 1, 2, 4, and 6). Thus, left main stem bronchus compression is consistently associated with anterior displacement of the thoracic aorta, but other central vessel anomalies and bronchomalacia also play a role in the causation of this disorder.[3-6]

Diagnosis of left bronchus compression is best achieved through a combination of endoscopy and MRI. Endoscopy allows visualization of the narrowed airway lumen, estimation of the degree of stenosis, and identification of any associated airway lesions. Furthermore, endoscopy affords a dynamic assessment of the airway during spontaneous ventilation. An MRI scan provides an assessment of the length, exact location, and degree of focality of the lesion, as well as identification of associated cardiovascular and pulmonary anomalies. If associated anomalies are found, further studies, including echocardiography, barium swallow study, cardiac catheterization, and pulmonary function testing, may be necessary. Several recent studies, as well as our own experience, make a convincing argument that any significant pediatric airway lesion merits MRI. In most of our cases, left main stem bronchus narrowing was not suspected on plain chest radiographs or fluoroscopy. By providing detailed images of the central vessels and airways, MRI greatly facilitates diagnosis of congenital vascular compression syndromes.[7-9]

Only two of our patients had symptoms secondary to compression of the left bronchus between the aorta and right, left, or main pulmonary artery accompanied by no other lesions (cases 7 and 8). Four of our patients had associated cardiovascular anomalies: two had tetralogy of Fallot, one also had an aberrant right subclavian artery, another patient had pulmonary artery sling and innominate artery compression, and patient 5 also had innominate artery compression. Two had associated intrinsic laryngotracheal anomalies: patient 4 had complete tracheal rings and bridging bronchus anatomy, and patient 3 had laryngomalacia and subglottic stenosis. Two patients had systemic diseases: patient 2 had DiGeorge's syndrome, and patient 5 had cystic fibrosis. Patient 6 had a very unusual fibrous ligament that extended from the ductus arteriosus to circumferentially envelop a segment of the left main stem bronchus. We are aware of no other cases of such a ligament. Overall, six of eight patients had associated cardiac, vascular, tracheal, or pulmonary anomalies, often with multiple lesions.

Treatment depends on the severity and anatomy of the lesion. Associated anomalies often need to be repaired. Four of our eight patients required surgical intervention that was directed at relieving left bronchus compression: patients 1 and 2 had repair of tetralogy of Fallot, with reconstruction of the pulmonary vessels to decrease their size, patient 6 had lysis of a fibrous band and posterior aortopexy, and patient 8 had aortic and pulmonary artery suspension. Two patients required surgery for associated airway lesions without repair of left main stem bronchus vascular compression: patient 4 had repair of a pulmonary artery sling and intrinsic tracheal anomalies, and patient 3 required tracheotomy because of laryngomalacia and subglottic stenosis. Patient 7 has not yet required any surgical intervention, but remains symptomatic. Patient 6 is improved, but remains symptomatic, 1½ years after surgical intervention.

Analysis of these patients suggests that left main stem bronchus compression by itself is amenable to observation in minimally to moderately symptomatic patients. With growth, the diameter and rigidity of the bronchial cartilages, as well as vessel positioning, may improve such that intervention is unnecessary, as is the case with innominate artery compression in most children. In patients with more severe symptoms, repair is needed. Repositioning of the descending aorta or pulmonary artery by suspension sutures, utilizing intraoperative endoscopic guidance to verify restoration of luminal patency, is the method of choice in left main stem compression.

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MARROW-MESENCHYME CONNECTIONS IN THE FETAL AND NEWBORN TYMPANUM

A NEW ENTITY

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Examinations of 41 human fetal, 8 infant, and 8 juvenile temporal bones prepared for light microscopic evaluation revealed direct connections between the hematopoietic bone marrow and the unresolved mesenchyme in the middle ear. The connections first appeared at 15 weeks of gestation and became bridged by fibrous tissue, in most cases, by the postpartum age of 10 months. Between 16 and 18 months after birth, the marrow-mesenchyme connections gradually disappeared. The areas in which the connections were most numerous were the anterior epitympanum, the sinus tympani medial to the stapedius muscle, and transitory bone that occupies the area that will become the aditus of the antrum. Immunohistochemical staining demonstrated the existence of mature leukocytes in these connections. These connections may help protect the middle ear against bacterial invasion during the postnatal period.

KEY WORDS — bone marrow, fetus, mesenchyme, newborn, otitis media.

INTRODUCTION

Otitis media, one of the most common diseases in infants and young children, occurs less frequently in the postnatal period than later in infancy.[1] A short and wide eustachian tube, tensor palatini muscle insufficiency, and immune deficiency have been described as possible etiologic agents in the development of infant otitis media. Although one or more of these factors are operational in the newborn, the incidence of infection is less common then than toward the end of the first year of life. The major difference between the newborn middle ear and that of the older infant is the presence of unabsorbed mesenchyme.

TABLE 1. CAUSE OF DEATH IN 57 CASES

Cause of Death	*No. of Cases*
Amniotic fluid infection syndrome	1
Breach delivery	2
Cardiovascular anomalies	7
Fetal hemorrhagic streptococcal pneumonia	1
Granulocytic leukemia	3
Hyaline membrane disease	10
Hypoplastic kidney	1
Hypoplastic lung	2
Kidney infarct	3
Leptomeningitis	1
Macerated stillborn	1
Multiple congenital anomalies	2
Placenta previa	1
Platinum poisoning	1
Polycystic kidney	1
Postmaturity	1
Preeclampsia	1
Prematurity	10
Prenatal rubella	1
Pulmonary atelectasis	1
Respiratory distress syndrome	2
Stillbirth (twin)	2
Trisomy 13	1
Trisomy 21	1
Total	57

Examination of fetal and newborn temporal bones in our laboratory revealed that in all cases, there are previously undescribed uninterrupted continuities of the hematopoietic bone marrow with the unresolved mesenchyme of the middle ear. We defined continuous links of leukocytes connecting the marrow and mesenchyme as "marrow-mesenchyme connections"

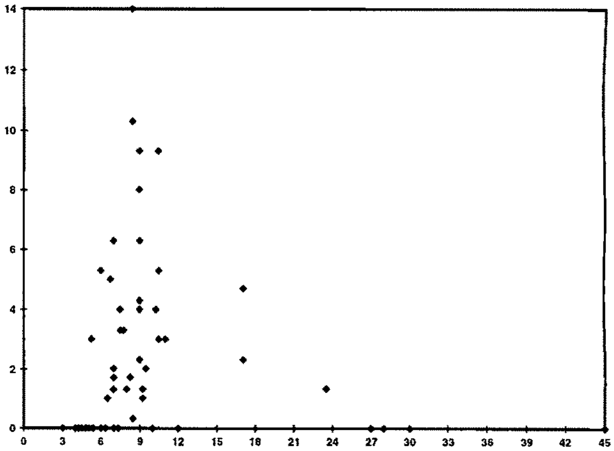

Fig 1. Frequency polygon shows number of marrow-mesenchyme connections (y-axis) related to different ages (months from conception; x-axis).

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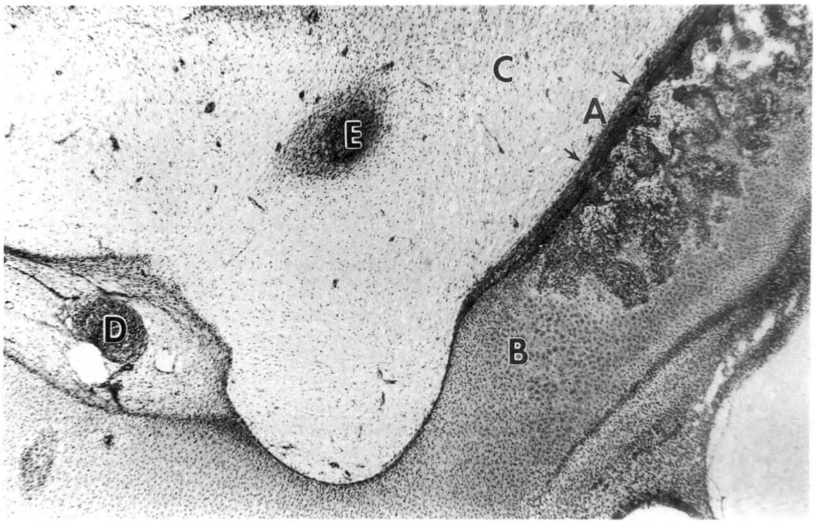

Fig 2. Marrow-mesenchyme connections (arrows) traversing periosteum (A) between primitive marrow in fetal intrachondrial bone (B) and middle ear mesenchyme (C) in sinus tympani of 15-week-old fetus (H & E, original ×40). D — facial nerve, E — tip of incus long process.

(MMCs). The MMCs are a normal developmental finding in the perinatal period. The MMCs are most common in the anterior epitympanum, medial to the tensor tympani muscles, in the tympanic recess inferior to the pyramidal process of the stapedius muscle, and in the vestigial bone of the aditus of the antrum, which will be absorbed as the mastoid air system matures. The presence of the MMCs in human newborn infant middle ears and with obvious outpouring of leukocytes into the lining of the middle ear suggests that the MMCs may be related to the low incidence of otitis media in the perinatal period.

We have performed a quantitative analysis of the frequency of MMCs at different stages of fetal and infant development, to demonstrate the relation of the MMC to the frequency of otitis media.

MATERIALS AND METHODS

Fifty-seven human fetal, infantile, and juvenile temporal bones prepared in our laboratory and others were examined with light microscopy. Each set of temporal bone sections consisted of 20 to 30 hematoxylin and eosin–stained slides. Cause of death is shown in Table 1. Clinical histories included gestational age measured in weeks or postnatal age measured in months or years, the interval between delivery and death, the interim between death and temporal bone removal, and the autopsy findings. We examined all of the sections in each case with light microscopy. Three sections from each bone, with complete middle ear structures including the tympanic membrane, ossicles, and the three areas where the MMCs are most commonly found (the anterior epitympanum, the vestigial aditus of the antrum, and the sinus tympani), were selected for evaluation. The numbers of MMCs for different stages of development were recorded (Fig 1).

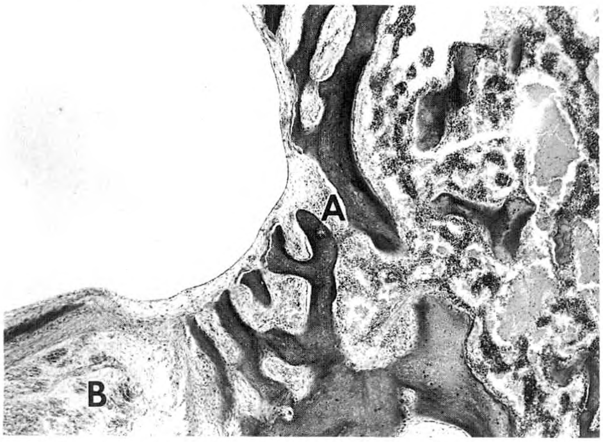

Fig 3. Marrow-mesenchyme connection (A) in tympanic recess of 23-week-old fetus (H & E, original ×40). Thickness of mesenchyme is diminished in this case. B — stapedius muscle.

In five bones, six or seven nonstained celloidin sections adjacent to the hematoxylin and eosin sections were stained with an immunohistochemistry technique using monoclonal anti–CD 15, a marker for granulocytic leukocytes, from BioGenex. The sections were removed from storage in 80% ethyl alcohol and run through serial dehydration with 95% and 100% ethyl alcohol to 100% methanol. The celloidin was removed by the method described in a previous paper.[2] After hydration with 100% methanol, 80% methanol, and phosphate-buffered saline (PBS), 0.03% Triton-X-100 was used to increase the permeability of the sections. Sections were submerged in 1:4 ficin solution and put into a 60°C oven for 15 minutes to break down the protein bond. The sections were rinsed with PBS two times for 10 minutes. Then casein PBS was used for 30 minutes to block the nonspecific staining. Sections were incubated in ready-to-use anti–CD 15 overnight at 4°C. On the second day, the sections were rinsed with casein PBS for 20 minutes and incubated with BioGenex supersensitive mouse link for 1 hour. Washed

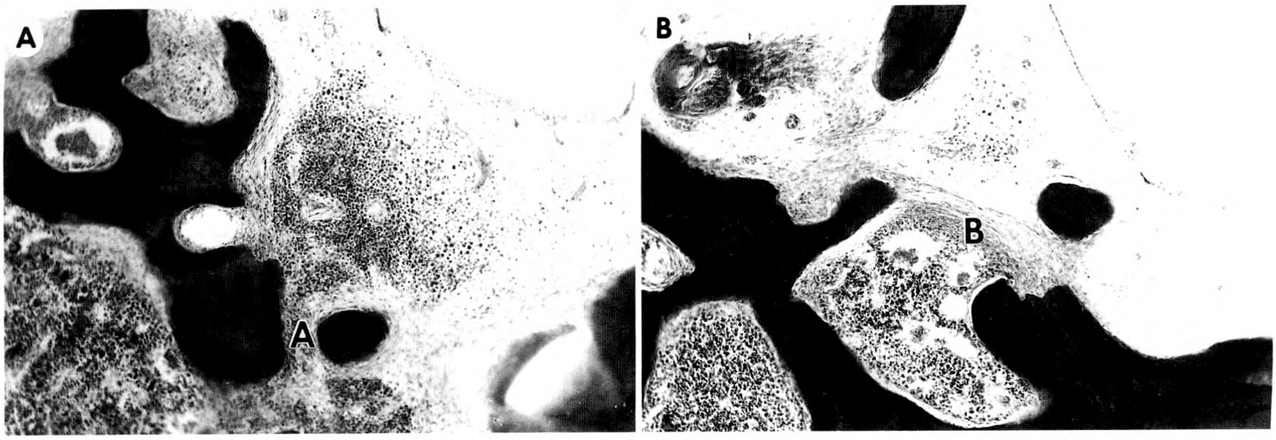

Fig 4. Original ×100. A) Marrow-mesenchyme connection (A) in anterior epitympanum of 10-month-old infant. B) Bridge of periosteum (B) across opening of previous marrow-mesenchyme connection in same area as A.

for 20 minutes in PBS, the sections were incubated for 1 hour in Vector standard ABC (avidin and biotinylated horseradish peroxidase macromolecular complex) solution. After washing in PBS, nickel diaminobenzidine solution was used to stain the slides. Eosin was used for counterstaining. After serial dehydration from ethyl alcohol to xylene, the sections were coverslipped with Permount.

RESULTS

The primary MMCs first appeared, at about 15 weeks of gestation, as dehiscences between the primitive marrow in the intrachondrial bone replacing the fetal cartilage (Fig 2). Later, at about 21 weeks of fetal development, after the intrachondrial bone and primitive marrow were replaced by endochondral bone and mature hematopoietic marrow, the connections became more evident (Fig 3). The mature connections increased in frequency from 21 weeks of gestation until birth, at which time CD 15–positive cells were visible in the middle ear and the number of MMCs began to decrease. A decrease in numbers of connections was first noticed when bridges of periosteum formed across the connection openings (Fig 4); by the age of 19 months, they were absent (Fig 5 and Table 2).

The MMCs, in all but the nonviable fetuses, exhibited the confluence of the marrow with the leukocytes in the mesenchyme. The continuity of the white cells in the marrow and those seen in the mesenchyme was demonstrated by the staining response to monoclonal anti–CD 15 (Fig 6).

Because the temporal bones examined in this study were from spontaneous abortions or newborns or children who died of various terminal conditions as shown in Table 1, most subjects had a terminal pneumonia with accompanying subclinical otitis media. The majority of the specimens in this report exhibited middle ear fluid containing a few leukocytes; only three had a fulminating otitis media manifested by a profuse accumulation of pus cells. In cases

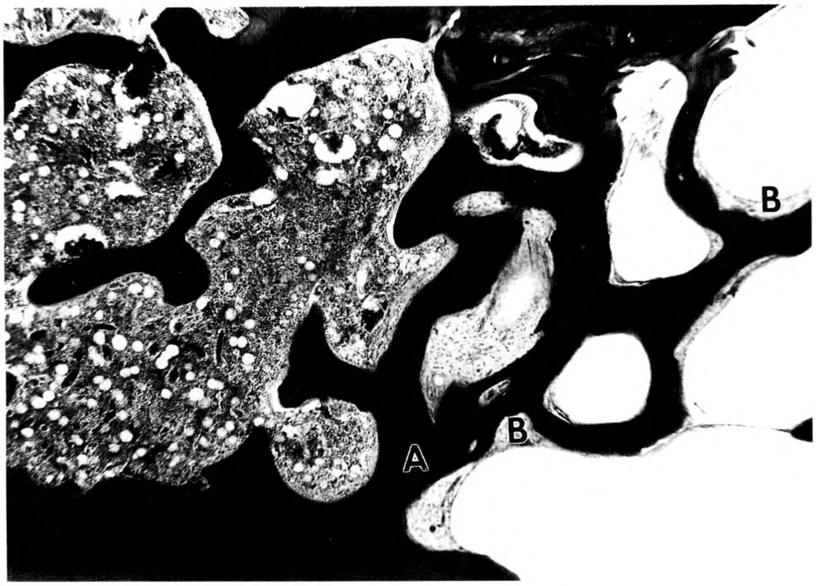

Fig 5. Periosteal bone (A) separating marrow from thinning mesenchyme (B) in vestigial bone in area that will be absorbed and result in space of mastoid antrum, in 18-month-old infant (H & E, original ×40).

TABLE 2. AGE DISTRIBUTION AND AVERAGE NUMBER OF MARROW-MESENCHYME CONNECTIONS

Gestational Age	*No. of Cases*	*Average No. of Connections*
12 wk	1	0
15 wk	8	0
20 wk	5	1.7
25 wk	9	1.9
30 wk	7	4.0
35 wk	11	4.8
40 wk	4	4.9
Birth to 6 mo	2	1.5
7 to 12 mo	2	3.5
13 to 18 mo	5	0.3
19 to 24 mo	1	0
>24 mo	2	0
Total	57	

of otitis media, mature leukocytes appeared to be passing directly from the hematopoietic marrow through openings in the bone into the unresolved middle ear mesenchyme (Fig 7).

DISCUSSION

The cartilaginous human fetal cochlear capsule reaches its full size by the 27th gestational week. The cartilage of the supporting bone of the cochlear capsule is replaced by primary intrachondrial bone with primitive bone and marrow and then by secondary endochondral bone containing mature bone marrow. After birth, due to the continuous remodeling of the bone, the amount of marrow decreases.[3] The portion of the cochlear capsule in contact with the middle ear area is covered by a thin periosteal bone layer that extends onto the surfaces of the surrounding bone of the middle ear. In the anterior epitympanum, the sinus tympani, and the vestigial bone of the developing antrum, there are gaps in the periosteal bone that result in direct connections between the bone marrow and the unresolved middle ear mesenchyme.

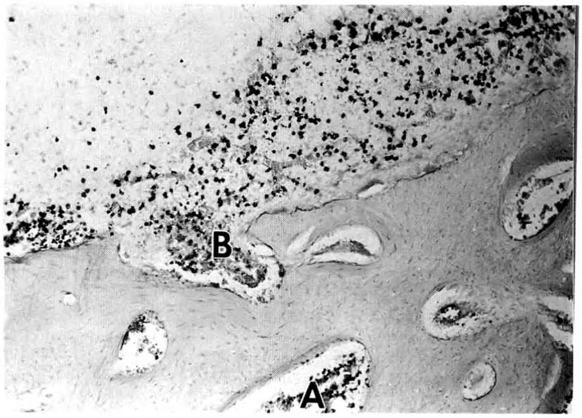

Fig 6. Monoclonal anti-CD stain of hematopoietic bone marrow (A), and cells in mesenchyme (B) demonstrating same staining response, in anterior epitympanum of term infant (×100).

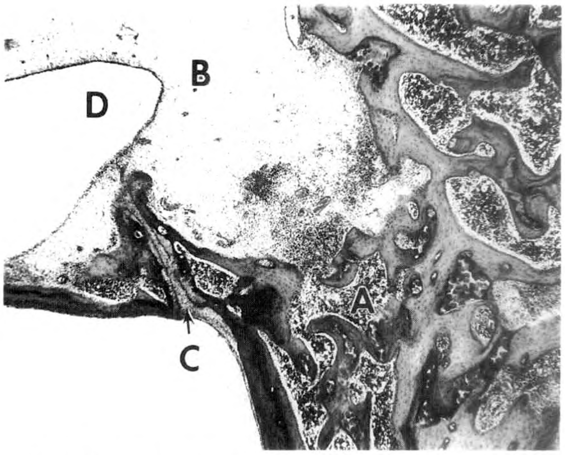

Fig 7. Marrow-mesenchyme connection in sinus tympani area of term infant demonstrating continuity of hematopoietic marrow (A) with unresolved middle ear mesenchyme (B) (H & E, original ×40). C — annular ligament of stapes, D — middle ear space.

The unresolved middle ear mesenchyme is a result of the failure of the middle ear to completely pneumatize before birth. The fetal middle ear is originally filled with loose mucoid connective tissue that is invaded by the expanding tympanic epithelium outpouching of the eustachian tube. The expansion of the tympanic epithelium continues until it surrounds the ossicles and enlarges the middle ear space that becomes filled with meconium via the eustachian tube. Bits of desquamated squamous epithelium, presumably from fetal skin, can be seen in the middle ear meconium. At birth, there is still a layer of unresolved

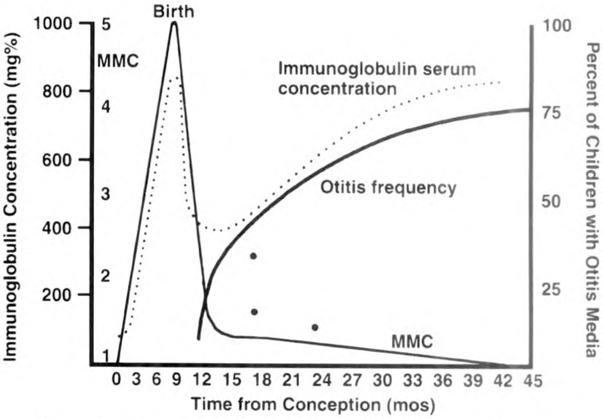

Fig 8. Relative number of marrow-mesenchyme connections (MMCs), mean serum immunoglobulin levels, and frequency of otitis media from conception to 3 years of age. Black dots represent cases with more than mean number of connections after birth. Adapted from Nelson et al[4] (immunoglobulin levels), Bluestone and Klein[5] (otitis media frequency), and our data (marrow-mesenchyme connections).

mesenchyme lining most of the middle ear space; in some areas — the anterior epitympanum, sinus tympani, and the vestigial bone of the antrum — it is thicker than in the rest of the middle ear.

The number of the connections exhibiting what appears to be movement of white cells between the marrow and the mesenchyme is highest around birth, and then there is a decrease in number to none by the age of 19 months. Also at birth, the serum immunoglobulin level is highest, as it is derived from the maternal circulation, before the infant system begins to manufacture its own, as reported by Nelson et al[4] (Fig 8). Coincidentally, the incidence of otitis media, as reported by Bluestone and Klein,[5] is lowest when the number of MMCs and the immunoglobulin levels are highest, in spite of contamination of the middle ear by meconium and foreign material. As the number of connections decreases, the incidence of otitis media increases in spite of an increase in the serum immunoglobulin level[5] (Fig 8). We suggest that the MMCs, as well as immunoglobulins from the maternal circulation, may be related to the low incidence of severe acute otitis media in the newborn.

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AURICULAR AND MIDDLE EAR MALFORMATIONS, EAR DEFECTS AND THEIR RECONSTRUCTIONS

The Third International Symposium on Auricular and Middle Ear Malformations, Ear Defects and Their Reconstructions will be held in Lubeck, Germany, September 6-9, 1997. For more information, contact Dr H. Weerda or Dr R. Siegert, Department for Otorhinolaryngology and Plastic Head and Neck Surgery, Medizinische Universitat zu Lubeck, Ratzeburger Allee 160, D-23538 Lubeck, Germany; telephone (0) 451 500 3189; fax (0) 451 500 4192.

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GIANT CHOLESTEATOMA OF THE EXTERNAL AUDITORY CANAL

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Cholesteatomas are found almost exclusively in the middle ear and mastoid. Occasionally this disease is seen in the external auditory canal. Cholesteatoma of the external auditory canal is a rare condition. Severe pain and profuse discharge associated with a normal eardrum and normal hearing are essential clinical features. In addition, we found facial paresis and conductive hearing loss in our case. Smaller cholesteatomas can be managed by frequent debridement in the office; larger lesions require surgical intervention. Surgery is successful in resolving otorrhea and relieving pain. In addition, our own experience has shown that surgery is successful in relieving facial paresis.

KEY WORDS — cholesteatoma, external auditory canal, facial paresis.

INTRODUCTION

Keratosis obturans and external auditory canal cholesteatoma (EACC) are two different clinical and pathologic processes. Piepergerdes et al[1] made a distinction between keratosis obturans and EACC. Keratosis obturans is caused by osteomas, stenosis of the canal, or hard masses of cerumen. Blockage of the external ear canal (EAC) for a long period permits epithelial debris to accumulate in the canal. This accumulation causes gradual expansion of the bony contour of the canal. In EACC the floor of the bony canal is eroded by localized accumulation of desquamated debris. When the EACC is large and reaches the annulus, the lesion erodes the middle ear and attic. Cholesteatoma of the EAC is a rare otologic entity, especially when keratosis obturans is excluded,[1,2] but has received much attention in the literature. This report will present a patient with giant cholesteatoma of the EAC.

CASE REPORT

A 40-year-old woman had a 15-year history of left otitis media, and progressive hearing loss and headaches. Five years previously a cholesteatoma was removed through a transcanal procedure at another institution. She was first seen in our clinic in May 1991 because of drainage of the left ear. There was a cholesteatoma of the medial superior canal, with hard necrotic bone and granulation tissue. The tympanic membrane, however, was intact. After repeated outpatient visits, the left ear was cleaned of the hard debris, revealing erosion of the canal wall. She was later seen in July 1991, the lesion having healed well and without further disease. When she was seen 3 years after her first visit, she had excessive drainage of the left ear, headaches, and left facial paresis. Left ear examination demonstrated a giant cholesteatoma in the auditory canal. Cholesteatoma extended to the mastoid and tympanic cavities, with extensive destruction in these areas.

Her audiogram demonstrated a speech reception threshold of 65 dB on the left, an air-bone gap of 40 to 50 dB, and a sloping high-frequency sensorineural hearing loss. Her discrimination score was 90%. A computed tomography scan was performed that showed that the posterior and anterior walls of the EAC of the left ear were eroded by an adjacent soft tissue mass. A mass extending into the tympanic cavity had eroded the inferior margin of the lateral wall of the attic, extended into the epitympanic space, and eroded the ossicles. A soft tissue mass was noticed on the tympanic segment of the fallopian canal and horizontal semicircular canal, which had eroded the fallopian canal. No erosion of the horizontal semicircular canal was detected (see Figure, A).

Intraoperatively, a large cavity was observed in the mastoid. Cholesteatoma was found to be adherent to the cavity and extending into the EAC (see Figure, B). The posterior canal was not observed. The tympanic segment of the fallopian canal was eroded, but did not invade the facial nerve (see Figure, C). The ossicles were totally engulfed by cholesteatoma. A silicone rubber sheet was placed in the tympanic cavity and the tympanic membrane was reconstructed

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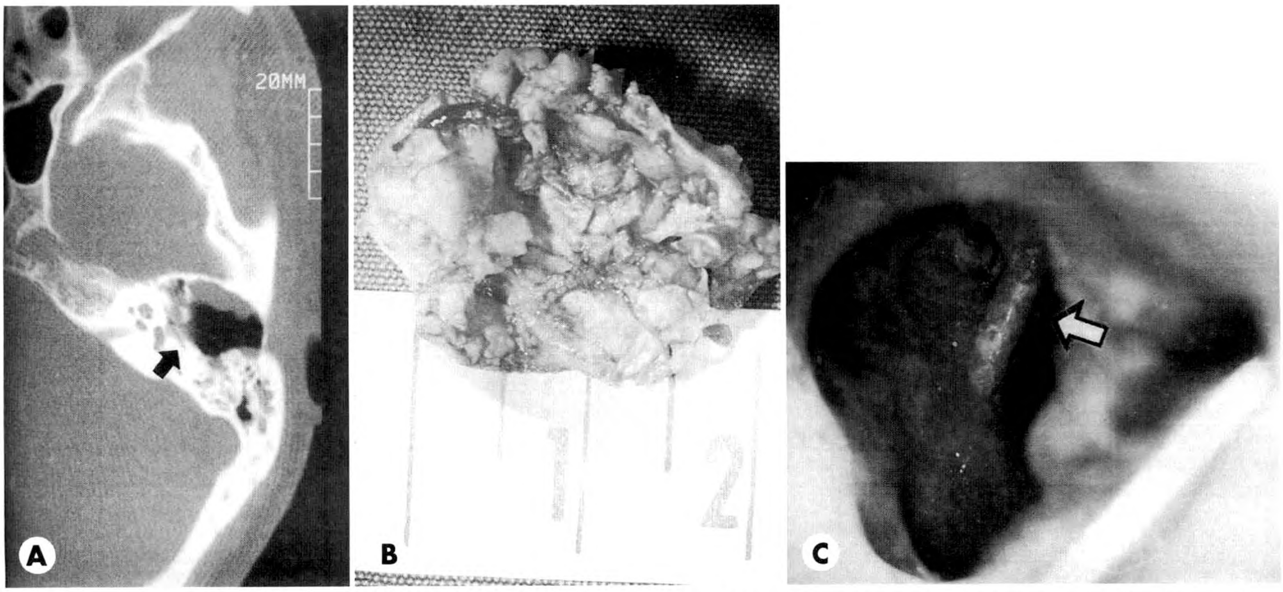

Giant cholesteatoma of external auditory canal. A) Axial computed tomogram shows that anterior and posterior walls of external auditory canal of left ear were eroded by adjacent soft tissue mass. Mass extending to tympanic cavity was noticed on tympanic segment of fallopian canal (arrow). B) Photograph of cholesteatoma. C) Intraoperative view of facial nerve (arrow) after complete removal of cholesteatoma.

with a temporalis fascia graft. The facial paresis and the cavity healed well, the graft remained intact, and there has been no evidence of recurrence 6 months after her operation. Postoperative audiologic examination revealed a 20-dB sensorineural hearing loss, a 35-dB conductive loss, and a speech discrimination score of 80%.

DISCUSSION

In EACC, the cause is unknown. Brown,[3] who reported the largest series, found no known cause. Bhide et al[4] believed that multiple trauma to the canal skin leads to chronic inflammation followed by invasion of cholesteatoma. Piepergerdes et al[1] reported that local periostitis of the canal bone is the primary cause of EACC. Holt[5] has divided the causes of EACC into five groups depending on the cause: 1) postsurgical, 2) posttraumatic, 3) ear canal stenosis, 4) ear canal obstruction, and 5) spontaneous.

Cholesteatoma of the EAC is most common in the age range of 40 to 75 years.[1,4-6] There have been no case histories in the literature of bilateral involvement. There is no clear association of EACC with sinusitis or bronchiectasis, as there is with keratosis obturans.[1,7,8] Our case supports these observations.

In EACC, the presenting symptoms are intermittent or persistent otorrhea and a dull, chronic otalgia.[1,5] Otorrhea and otalgia were the presenting symptoms in our case of EACC. Hearing loss due to the blockage of the EAC with keratin debris is the presenting symptom in keratosis obturans.[1] In EACC, in contrast, hearing loss due to the cholesteatoma does not occur, because the tympanic membrane is normal.[1,4,5] However, in our case, hearing loss due to the cholesteatoma occurred because the cholesteatoma had eroded the canal wall, attic, tympanic membrane, and middle ear. Also, there have been no case histories in the literature of involvement of the fallopian canal. In our case, the cholesteatoma had eroded the canal wall and reached the tympanic cavity, with extensive destruction in this area. It had eroded the fallopian canal, but did not invade the facial nerve. Finally, facial paresis was also seen.

The cause in our case is unknown. The patient had no history of surgery, trauma, ear canal stenosis, or obstruction.[5] Our theory is that local periostitis of the canal bone was the cause of the cholesteatoma.

Treatment options can be divided into three categories depending on the stage of the disease.[5] For localized, small lesions, frequent cleaning with debridement of necrotic tissue is recommended. Deeper pockets can be managed with canaloplasty by removing diseased skin and bone. For mastoid involvement, mastoid surgery is needed. In our case, a postauricular approach and mastoid surgery was performed. Our own experience and those of Piepergerdes et al,[1] Bhide et al,[4] and Holt[5] have all shown that surgery is successful in resolving otorrhea and relieving pain. In addition, our own experience has shown that surgery is successful in relieving facial paresis.

CONCLUSIONS

Cholesteatoma of the EAC is a rare clinical entity, with severe pain, profuse discharge associated with a normal eardrum, and normal hearing being essential clinical features. In addition, we found facial paresis and conductive hearing loss, although these findings are not generally present. Smaller cholesteatomas can be managed by frequent debridement in the office; larger lesions require surgical intervention. The rapid response to surgery is dramatic and rewarding.

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1997 CONFERENCE ON IMPLANTABLE AUDITORY PROSTHESES

The 1997 Conference on Implantable Auditory Prostheses will be held August 17-21, 1997, in Pacific Grove, California. See the Conference World Wide Web page (http://www.rti.org/ciap97) for more information, or contact Alena Wilson, Conference Coordinator, House Ear Institute, 2100 W Third St, Los Angeles, CA 90057; telephone (213) 353-7086; fax (213) 413-0950; email: alena@hei.org.

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DECREASE IN IMMUNOREACTIVE NEUTRAL ENDOPEPTIDASE IN UVULA EPITHELIUM OF PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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The purpose of this study was to determine whether neutral endopeptidase (NEP; EC 3.4.24.11) is decreased in the uvula epithelium of patients with obstructive sleep apnea (OSA). Tissues were obtained by uvulopharyngopalatoplasty in seven patients with moderate OSA and by autopsy in five individuals not known to have OSA. Using antisera to human NEP and immunoperoxidase staining, we found that NEP was localized in uvula epithelial cells of both patients with OSA and controls. However, there was a significant decrease in the number of epithelial cells staining for NEP in patients with OSA relative to controls (67 ± 10 cells versus 261 ± 33 cells, in 5 randomly selected high-power microscopic fields, respectively; mean ± SEM; $p < .05$). The intensity of staining for NEP was similar in both groups. We conclude that immunoreactive NEP is significantly decreased in the uvula epithelium of patients with OSA.

KEY WORDS — CALLA, CD10, immunohistochemistry, inflammation, soft palate, uvulopharyngopalatoplasty.

INTRODUCTION

Obstructive sleep apnea (OSA), a relatively common disorder in the United States, is characterized by recurrent episodes of upper airway obstruction during sleep leading to significant hypoxemia and cardiovascular complications.[1-3] Current concepts suggest that anatomical and functional abnormalities play a role in modulating upper airway obstruction in these patients.[3-8]

A major site of this obstruction is the soft palate.[2-4,8] Sekosan et al[9] recently showed that inflammation, characterized by interstitial edema and mononuclear cell infiltration, is present in the soft palate of patients with OSA. However, the mechanisms underlying this process and its role in modulating upper airway obstruction during sleep are uncertain.

It is well established that the epithelium modulates lower airway caliber in diseases associated with airway inflammation, such as asthma, chronic bronchitis, and cystic fibrosis.[10-14] For instance, down-regulation of neutral endopeptidase (NEP; EC 3.4.24.11), a ubiquitous ectoenzyme that cleaves and inactivates proinflammatory peptides that elicit interstitial edema, such as bradykinin and substance P,[15,16] in the airway mucosa, is associated with airway hyperreactivity to various environmental irritants.[10,12,14]

The role of the oropharyngeal epithelium in modulating upper airway patency in patients with OSA is uncertain. The purpose of this study was, therefore, to begin to address this issue by determining whether immunoreactive NEP is decreased in the uvula epithelium of patients with OSA.

METHODS

Subjects. We studied seven nonsmoking patients (36 ± 5 years of age; five men and two women) with OSA (apnea/hypopnea index, 31 ± 6) who underwent uvulopharyngopalatoplasty and five autopsied individuals not known to have OSA (43 ± 6 years of age; three men and two women; three smokers and two lifelong nonsmokers) who died of causes unrelated to upper airway disease. Patients with OSA denied having acute or chronic upper or lower airway disorders and were not taking anti-inflammatory medications for at least 6 months before surgery.

Tissues. Tissues removed at surgery and autopsy were immediately placed in 10% neutral buffered formalin solution and embedded in paraffin by standard histologic methods. The samples were oriented transversely and serially sectioned. Four-micron tissue sections were prepared from each paraffin block. One deparaffinized 4-µm-thick section was stained with hematoxylin and eosin to determine tissue morphology. Other tissue sections were used for immunohistochemistry stains as outlined below.

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NEUTRAL ENDOPEPTIDASE IMMUNOREACTIVITY IN UVULA EPITHELIUM

	No. of Epithelial Cells Stained per Five High-Power Microscopic Fields	*Intensity of Staining*
OSA patients		
1	83	3
2	90	3
3	69	1
4	61	1
5	94	1
6	16	1
7	59	2
Mean ± SEM	67 ± 10	1.7 ± 0.4
Controls		
1	290	2
2	237	2
3	347	3
4	147	2
5	283	3
Mean ± SEM	261 ± 33*	2.4 ± 0.2

OSA — obstructive sleep apnea.
*$p < .05$ in comparison to OSA patients.

Immunohistochemistry. Antisera to purified human kidney NEP were obtained in rabbits as previously described by Erdös and Skidgel[16] and Dragović et al.[17] They were purified on a protein A column to obtain IgG fraction. This fraction was diluted and filtered through Spin-X centrifuge microfilters (Costar, Cambridge, Mass) before staining tissue sections. Tissue sections were deparaffinized in xylene and through graded alcohols to water. Endogenous peroxidase activity was blocked by incubating the tissue slides in methanol and 0.3% hydrogen peroxide for 30 minutes at room temperature. After the slides were washed with phosphate-buffered saline (Gibco BRL, Grand Island, NY), they were incubated with normal goat serum (Kirkegard & Perry Laboratories, Gaithersburg, Md) for 30 minutes at room temperature to block nonspecific binding. Then, they were covered with rabbit IgG against human NEP or, as controls, with rabbit anti-NEP IgG preadsorbed with human recombinant NEP (Khepri Pharmaceuticals, South San Francisco, Calif) and normal rabbit serum IgG and incubated overnight at 4°C. The sections were then washed with phosphate-buffered saline, and biotinylated goat anti rabbit antibody was applied for 1 hour at room temperature. After rinsing with phosphate-buffered saline, the sections were covered with a streptavidin peroxidase complex (Kirkegard & Perry Laboratories) and incubated for 30 minutes at room temperature, washed once more, and incubated with diaminobenzidine substrate for 5 minutes. Thereafter, the slides were washed, counterstained with hematoxylin, and mounted with Permount (Fisher, Fair Lawn, NJ) on coverslips.[18] Human kidney sections were used as a positive control for NEP.[17]

Data and Statistical Analyses. Sections were examined at ×100 magnification with an Olympus microscope. Five microscopic fields were randomly selected in each section, and the total number of squamous epithelial cells staining positive for NEP were counted by an observer unaware of the tissue origin. In addition, the maximal intensity of NEP staining in the five microscopic fields was graded on a scale of nil (no detectable staining) to 3 (intense staining). All values are expressed as mean ± SEM. Statistical analysis was performed by analysis of variance and the Newman-Keuls test for parametric data or by the Mann-Whitney test for nonparametric data. A p value of $<.05$ was considered significant.

RESULTS

The intensity of immunoperoxidase staining of uvula squamous epithelial cells for NEP was similar in patients with OSA and controls (see Table; $p > .1$). In both groups, basal and more superficial epithelial cell layers were stained positive for NEP. Sections incubated with rabbit anti-NEP IgG preadsorbed with human recombinant NEP or with normal rabbit serum IgG showed no immunoperoxidase staining.

The total number of uvula squamous epithelial cells staining positive for NEP in five high-power microscopic fields was less in each patient with OSA relative to controls (see Table). As a group, there was a fourfold decrease in the total number of cells staining positive for NEP in patients with OSA relative to controls (see Table; 67 ± 10 versus 261 ± 33 cells, respectively; $p < .05$).

DISCUSSION

The results of the present study show that immunoreactive NEP is significantly decreased in the uvula epithelium of patients with OSA relative to controls. We suggest that this phenomenon plays a role in modulating oral mucosa inflammation in these patients by slowing local catabolism of proinflammatory peptides cleaved and inactivated by NEP.

Histopathologic changes in the soft palate of patients with OSA have been previously reported.[5,6,8,19] For instance, Woodson et al[19] and Edström et al[6] showed that myelinated nerves and striated muscle are damaged in the uvula of these patients. Woodson et al[19] also noted inflammatory changes in the uvula consisting of edema and vascular congestion and dilation in the lamina propria. Stauffer et al[5] showed that the uvula of patients with OSA contained more striated muscle and fat than the uvula of controls.

Sériès et al[8] showed that protein content and anaerobic enzyme activities in the striated muscle in the uvula were significantly greater in patients with OSA relative to nonapneic snorers. The results of the present study support and extend these observations by showing that the uvula epithelium is altered in patients with OSA, as manifested by a significant decrease in immunoreactive NEP. Further studies are indicated to determine the mechanisms that mediate the decrease in immunoreactive NEP in the uvula epithelium of patients with OSA.

Neutral endopeptidase, a membrane-bound metalloenzyme widely distributed in epithelial cells of the airway, gut, and other organs,[16] has been recently shown to modulate plasma exudation and vasodilation elicited by bradykinin and substance P in the oral mucosa.[20-22] Rubinstein[23] showed that bradykinin concentrations are increased in the nasal lavage fluid obtained from patients with OSA relative to controls. It is conceivable that any decrease in NEP in the uvula epithelium could amplify tissue inflammation by slowing local catabolism of bradykinin, substance P, and other pro-inflammatory peptides cleaved and inactivated by the peptidase.[24-27] This, in turn, may amplify vasodilation and interstitial edema in the uvula mucosa, leading to an increase in upper airway collapsibility and a decrease in upper airway caliber. Support for our hypothesis comes from the study of Wasicko et al,[28] who showed that local vasodilation increases upper airway collapsibility and decreases pharyngeal cross-sectional area in anesthetized cats. The decrease in pharyngeal size was related mostly to increased thickness of the pharyngeal mucosa. Clearly, additional studies are warranted to support or refute this hypothesis.

In summary, we found that immunoreactive NEP is significantly decreased in the uvula epithelium of patients with OSA. We suggest that this phenomenon plays a role in modulating oral mucosa inflammation in these patients by slowing local catabolism of pro-inflammatory peptides cleaved and inactivated by NEP.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

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MIDDLE EAR PRESSURE AND DYSFUNCTION OF THE LABYRINTH: IS THERE A RELATIONSHIP?

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Relationships between middle ear pressure and non–infection-related cochleovestibular dysfunction have been suggested by several authors. According to some data, vertiginous attacks can be prevented by the insertion of a ventilation tube in patients suffering from Meniere's syndrome. The aim of our study was to investigate if the incidence of eustachian tube malfunction and pathologic middle ear pressure is frequent, and if routine implantation of ventilation tubes is reasonable in ears with dysfunctions of the labyrinth, including clinical Meniere's syndrome. So, we determined in our pressure chamber all active and passive parameters of eustachian tube function in 40 patients suffering from Meniere's syndrome, sudden sensory hearing impairment (SSHI), or vestibular neuronitis. Our results disclosed *no* nonrandom incidence of impaired tubal function among our patients compared to healthy control subjects. Pressure equalization was sufficient in most patients suffering from clinical Meniere's syndrome, and only one patient with vestibular neuronitis presented with a patulous tube. Our results show that impairment of vestibular or cochlear function is *not* regularly accompanied by eustachian tube dysfunction. Furthermore, no patient reported symptoms while pressure variation was performed. We conclude that variation of middle ear pressure does not usually play a role in the genesis of Meniere's syndrome, vestibular neuronitis, or SSHI. Thus, from our data, we cannot recommend routine implantation of tympanic ventilation tubes in patients suffering from Meniere's syndrome, vestibular neuronitis, or sudden hearing loss.

KEY WORDS — eustachian tube, Meniere's syndrome, sudden hearing impairment, ventilation tube, vestibular neuronitis.

INTRODUCTION

A relationship between middle ear pressure and non–infection-related dysfunction of the labyrinth has been suggested by several authors. Data published by Montandon et al[1] indicate that vertiginous attacks may be prevented by the insertion of a ventilation tube in patients suffering from Meniere's syndrome. Densert et al[2,3] and Ingelstedt et al[4] described relief of symptoms in Meniere's attacks by reduction of atmospheric pressure. Robinson and Hazell[5] observed cases of sudden sensory hearing impairment (SSHI) or vestibular lesions in patients presenting with a patulous tube.

Since many studies of possible interactions between middle ear pressure and inner ear dysfunction are deficient in methodology or are restricted to case reports, we started a prospective investigation on middle ear pressure in patients suffering from clinical Meniere's disease, vestibular neuronitis, or SSHI. The aim of our study was to investigate whether there actually are relationships between middle ear pressure and dysfunctions of the labyrinth.

MATERIALS AND METHODS

Forty subjects suffering from sudden disorders of the labyrinth were examined in our eustachian tube laboratory. The patients were classified into three groups according to the clinical symptoms. Fifteen patients presenting with the typical Meniere's triad (vertigo, sudden low-frequency hearing impairment, and tinnitus) formed the first group. The second group consisted of 15 patients with sudden high-frequency or pan-tonal sensory hearing impairment. The third group consisted of 10 patients with vestibular neuronitis. Histories were recorded and all patients were examined clinically and by pure tone and speech audiometry. Tympanometry, stapedius reflex test, brain stem evoked response audiometry, and serologic tests for borreliosis and lues were performed as well. Only patients without any history or clinical sign of infectious, traumatic, hereditary, or retrocochlear ear disease were included in the study. Furthermore, only patients presenting without a history or any symptoms indicating perilymphatic fistula (positional nystagmus, nystagmus following variation of ear canal pressure) were included. In addition, a group of 12 normal-hearing healthy subjects without a history of vertigo or hearing impairment was examined as a control.

Eustachian tubes were examined by the pressure

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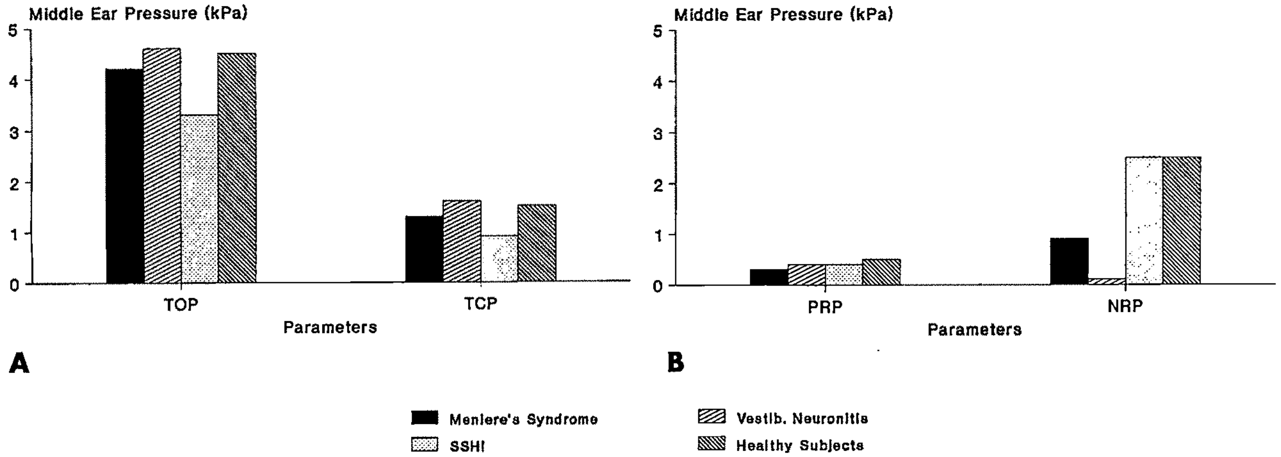

Mean values of tubal parameters in 40 patients suffering from clinical Meniere's syndrome, sudden sensory hearing impairment (SSHI), or vestibular neuronitis and in 24 healthy ears. A) Mean values of tubal opening pressure (TOP) and tubal closing pressure (TCP) during reduction of ambient pressure in chamber. Tendency toward lower values in cases with sudden deafness was not statistically significant on Student's *t*-test. B) Mean values of positive residual pressure (PRP) in tympanic cavity after reduction of ambient pressure and attempt at equalization by swallowing, and negative residual pressure (NRP) after elevation of ambient pressure and repeated swallowing. Comparatively low mean values in ears with Meniere's syndrome and vestibular neuronitis had no statistical significance on Student's *t*-test.

chamber impedance method as described by Münker.[6] Briefly, a rubber plug connected to a probe with three boreholes was inserted in each ear canal of the patient sitting in the pressure chamber. One borehole contained a loudspeaker (220 Hz), another contained a microphone, and the third hole was open, ensuring equalization of the pressure in the ear canal with that in the chamber. The microphone registered the sound reflected by the tympanic membrane, indicating the impedance of the middle ear. The impedance and air pressure in the chamber were recorded simultaneously.

We examined both eustachian tubes of each subject simultaneously, altering ambient air pressure in the pressure chamber (dynamic examination). First, passive tubal function was examined, ie, passive opening parameters of the tube in a state of overpressure in the middle ear. The chamber pressure was diminished continuously by 13 kilopascals (kPa) at a rate of 10 kPa/min. The initial pressure in the middle ear was recorded at the beginning. Then, tubal opening pressure (TOP), corresponding to the positive pressure in the middle ear forcing up the tube passively for the first time, and tubal closing pressure (TCP), describing the tubal resistance against a positive pressure in the middle ear, were analyzed. Second, we examined active tubal function, ie, the ability of the tube to equalize overpressure and underpressure in the middle ear by swallowing. All subjects were instructed to swallow once at the end of the phase of diminishing chamber pressure, and the residual overpressure (positive residual pressure; PRP) in both ears after swallowing was recorded. Finally, the ambient air pressure was adjusted to the normal atmospheric level at a rate of 10 kPa/min, with the patient equalizing negative middle ear pressure by repeated swallowing. Residual underpressure (negative residual pressure; NRP) in the middle ear after repeated swallowing was recorded. A statistical comparison between affected and healthy ears in each of the patient groups, as well as between affected ears of the patient groups and healthy ears of the control group, was performed for the parameters described above by means of Student's *t*-test.

In a supplementary test, tympanic membrane compliance was recorded while the subjects performed the following maneuvers: quick nasal breathing, sniffing (ie, quick and repeated inhalation through the nose), and Valsalva's maneuver (static tubal examination). The chamber pressure was not varied during static tubal examination.

RESULTS

Dynamic Examination of Tube. The initial middle ear pressure was within a range of ±0.5 kPa in all healthy and affected ears of the patients and in all ears of the control group. When comparing the mean values of passive tubal parameters (TOP and TCP) measured in 40 affected ears in the three patient groups and 24 healthy ears, we did not observe an increased incidence of impaired tubal function among our patients. In two groups (Meniere's syndrome and SSHI), the passive tubal parameters seemed to be even better than in healthy persons, but this tendency was not of statistical significance on the unpaired *t*-test. There was also no accumulation of cases with pathologic patency of the tube. Only 1 patient suffering from vestibular neuronitis presented with a patulous tube (on the affected side), in agreement with zero levels for TOP and TCP. Mean values of TOP and TCP are shown in the Figure, A. The comparison

EUSTACHIAN TUBE PARAMETERS AMONG THREE PATIENT GROUPS AND HEALTHY CONTROLS

Parameter		*Ear*	*MS (n = 15)*	*VN (n = 10)*	*SSHI (n = 15)*	*HS (n = 12)*
Passive	TOP	Affected	4.2 ± 1.3	4.6 ± 2.2	3.3 ± 1.5	
		Nonaffected	3.8 ± 1.3	4.9 ± 0.1	3.5 ± 1.5	4.5 ± 2.4
	TCP	Affected	1.3 ± 0.6	1.6 ± 1.6	0.9 ± 0.6	
		Nonaffected	1.3 ± 0.4	2.2 ± 1.4	1.3 ± 0.7	1.5 ± 1.1
Active	PRP	Affected	0.3 ± 0.2	0.4 ± 0.6	0.4 ± 0.2	
		Nonaffected	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.5	0.5 ± 0.6
	NRP	Affected	0.3 ± 0.9	0.1 ± 0.2	2.5 ± 3.6	
		Nonaffected	1.4 ± 3.0	0.9 ± 2.4	2.0 ± 3.5	2.5 ± 4.3

Data are in kilopascals (mean ± SD). Of patient groups, values of contralateral, nonaffected ears are figured in lower line. Statistical evaluation was performed on data of affected ears from each patient group and unaffected ears from same group (paired *t*-test) and revealed no significance ($p > .05$). Additional evaluation of affected ears from each patient group in comparison with total of 24 ears from control group (unpaired *t*-test) showed no statistical significance, either.

TOP — tubal opening pressure, TCP — tubal closing pressure, PRP — positive residual pressure, NRP — negative residual pressure, MS — Meniere's syndrome, VN — vestibular neuronitis, SSHI — sudden sensory hearing impairment, HS — healthy subjects.

of affected ears to healthy ears in any of the groups did not show a significant difference of the values, either, as proven by paired *t*-test. Mean values and statistical data of all examinations are presented in the Table.

Excellent active pressure equalization was found in all but 1 of the ears with Meniere's syndrome and all ears with vestibular neuronitis, expressed by lower mean values of NRP than those of 24 healthy ears, but an unpaired Student's *t*-test did not reveal any statistical significance. The mean values of PRP in all patient groups and NRP in the sudden deafness group were similar to those in 24 healthy ears. When comparing affected and healthy ears within each patient group, we observed no striking or even significant (paired *t*-test) difference. The results are shown in the Figure, B, and the statistical examination in the Table.

Static Examination of Tube. Valsalva's maneuver was positive in both ears of 35 patients and of 10 subjects of the control group. Excessive nasal breathing and sniffing resulted in alterations of compliance in only 1 ear, which was the ear presenting with a patulous tube in the dynamic examination. No patient reported vertigo during any of these maneuvers.

DISCUSSION

In a study of 28 patients suffering from Meniere's syndrome, Montandon et al[1] reported that vertiginous attacks could be prevented in 20 cases and reduced in 3 cases by the insertion of tympanic ventilation tubes. Hearing loss could not be reduced. The treatment failed completely in 5 patients, 4 of whom presented with advanced Meniere's syndrome with severe hearing loss, and 1 of whom suffered from drop attacks. The above treatment had originally been introduced by Tumarkin[7] in 1966, but his positive results could not be reproduced by Hall and Brackmann.[8] Lall[9] discussed the high rate of spontaneous remission in Meniere's syndrome as being responsible for the effects in Tumarkin's study. There are three possible reasons for a positive influence of tympanic ventilation tubes on the clinical course of Meniere's syndrome. First, dysfunction of active capacities of the tube, in particular the equalization of underpressure in the middle ear, could lead to phases of underpressure in the middle ear and consecutive impairment of labyrinthine function by pressure transmission through the round window. This hypothesis was introduced by Tumarkin[10] in 1961. A second putative reason was favored by Robinson and Hazell,[5] who reported cases of cochleovestibular dysfunction in patients with a patulous eustachian tube. In this circumstance, nasopharyngeal pressure variations could be directly transmitted to the middle ear, and possibly to the labyrinth by the round window as well. A third aspect is a placebo effect that may be caused by the fact that some therapeutic effort (eg, the insertion of grommets) has been done, and by the knowledge that the disease is neither dangerous nor life-threatening.[11] In this investigation we tried to find out if there is a predominance of tubal dysfunction that makes the regular insertion of ventilation tubes in Meniere's syndrome and other cochleovestibular disorders reasonable.

The results of our study showed no significant difference of tubal function in ears with cochlear or vestibular disorders when compared to the contralateral, unaffected ears or in comparison to the ears of healthy subjects. In our study the dual impedance method introduced by Münker[6] in 1972 was used for determining tubal function. Results of tubal parameters obtained by this method are very reproducible.[12,13] Values measured this way may be regarded as specific for the individual eustachian tube unless acute middle ear inflammation, weight loss, or a hormonal change interferes.[6]

We could not find any indication that impaired tubal function causing persistent overpressure or underpressure in the middle ear is a frequent concomitant factor in cochleovestibular impairment. On the other hand, only 1 of 40 patients presented with a patulous tube (which permits nasopharyngeal pressure variations to spread directly to the middle ear without restrictions[14]). This is within the frequency range of patulous tube syndrome among a normal population of healthy ears.[6] Thus, the hypothesis of Robinson and Hazell[5] that a patulous tube is a risk factor for cochleovestibular impairment is not supported by our investigation, either. In addition, no patient reported symptoms following Valsalva's maneuver, sniffing, or quick nasal breathing. We conclude from our results that regular insertion of ventilation tubes in patients with clinical Meniere's syndrome, vestibular neuronitis, or SSHI cannot be recommended.

Nevertheless, there are several reports indicating that in certain circumstances middle ear pressure *may* influence the labyrinth. Ingelstedt et al[15] reported that so-called "alternobaric vertigo" and nystagmus are induced in the case of severe unilateral tubal opening impairment during the reduction of atmospheric pressure. Densert et al,[2,3] Ingelstedt et al,[4] and Kitahara et al[16] reported a relief of Meniere's symptoms during hypobaric atmospheric pressure in a pressure chamber. Recent studies[17-19] show that both bone conduction levels and otoacoustic emissions amplitudes may be significantly reduced by underpressure in the middle ear in normal-hearing subjects.

Thus, we do not deny that ventilation tubes may have a preventive effect in some carefully selected patients suffering from Meniere-like symptoms that are not primarily based on cochleovestibular dysfunction. When reporting that ventilation tubes were successful in 2 patients suffering from vertigo, tinnitus, and hearing loss after descent from a mountain, Montandon et al[1] recommended the insertion of ventilation tubes in subjects suffering from typical barotrauma of the inner ear arising from insufficient eustachian tube function. This example from their publication implies that their definition of Meniere's syndrome was vague. We believe that this vague definition of Meniere's syndrome, a placebo effect,[11] and the high spontaneous remission rate of real Meniere's disease appear as the positive effects of a ventilation tube in the report of Montandon et al. Thus, the evaluation of the patient's history and the examination of the middle ear must be very exact. If alternobaric vertigo caused by persistent overpressure in one ear after ascent is present,[15] or if there are signs of underpressure that actually may cause temporary low-frequency sensory hearing loss,[17] ventilation tubes can prevent recurrence. If a patient's history indicates barotrauma, or if clinical signs of barotrauma are present (positional nystagmus, nystagmus following alteration of ear canal pressure), tympanotomy and inspection of the round and oval windows have to be discussed.[20]

We conclude that the insertion of transtympanic ventilation tubes may be of benefit to some patients presenting with cochleovestibular dysfunction if their history indicates a malfunction of the eustachian tube as the underlying factor. In order to select eligible patients, an exact evaluation of history and examination with special respect to the middle ear and eustachian tube is reasonable.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria (in English July 3-5, 1997, and in German September 4-6, 1997). For more information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax 43/316/385/3425.

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SIGNIFICANT CORRELATION BETWEEN SYMPTOM SCORE AND IGG4 ANTIBODY TITER FOLLOWING LONG-TERM IMMUNOTHERAPY FOR PERENNIAL ALLERGIC RHINITIS

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Although there is evidence of some measure of clinical benefit as well as immunologic change during the early phase of immunotherapy, a sustained clinical response is only possible with prolonged therapy. Immunotherapy has to be administered for about 3 to 5 years for such sustained clinical efficacy. This study aimed at investigating the dynamics of IgE and IgG4 antibodies after more than 5 years of immunotherapy, to examine the statistical correlation between these antibodies and symptom scores. Our study demonstrated that the allergen-specific IgE antibody level significantly decreases and the IgG4 antibody level significantly increases following immunotherapy. However, the percent decrease in IgE antibodies did not correlate with the percent decrease in symptom scores. On the other hand, the percent increase in IgG4 antibodies correlated with the percent decrease in symptom scores. We infer that an elevation of IgG4 antibodies is not simply an epiphenomenon unrelated to the underlying working mechanism of clinically successful immunotherapy, but probably makes an active contribution to symptom relief.

KEY WORDS — allergic rhinitis, IgE antibody, IgG4 antibody, immunotherapy.

INTRODUCTION

The house dust mite, *Dermatophagoides farinae*, has been indicated as one of the main sources of house dust allergen and is generally considered to be a major allergen associated with perennial allergic rhinitis in many countries. Allergen-specific immunotherapy has been widely used in the treatment of allergic diseases for more than 80 years, but still, in the opinion of many physicians, represents a controversial treatment of perennial allergic rhinitis. Critics of immunotherapy for perennial allergic rhinitis often claim a lack of documented efficacy. However, three recent double-blind placebo-controlled trials for perennial allergic rhinitis have all clearly shown the efficacy of immunotherapy.[1-3]

However, the major mechanisms by which this therapeutic method exerts its action in improving patient symptoms have been the subject of much debate and still remain controversial. Allergen-specific IgE and IgG4 antibodies are of key importance in allergic rhinitis. Considerable attention has been devoted to the role of IgE and IgG4 antibodies in relation to clinical outcome during the last decade. A review of the literature available indicates that the IgE antibody response shows an initial increase followed by a gradual decrease after several years of immunotherapy[4-7] and that IgG4 levels increase considerably during immunotherapy.[3,7-9] However, a role of IgG4 antibodies in the clinical efficacy of immunotherapy has yet to be established. Although there is evidence of some measure of clinical benefit as well as immunologic change during the early phase of immunotherapy, a sustained clinical response is only possible with prolonged therapy. Immunotherapy has to be administered for about 3 to 5 years for such sustained clinical efficacy.[10] To the authors' knowledge, however, no data can be found in the literature concerning the dynamics of IgG4 antibodies following long-term immunotherapy. We have therefore investigated the dynamics of allergen-specific IgE antibodies and IgG4 antibodies in patients who were receiving immunotherapy for more than 5 years, with special reference to symptomatic relief.

MATERIALS AND METHODS

Patients. This study included 48 patients in the age range 13 to 61 years who had variable periods of long-term immunotherapy with Hollister-Stier standardized *D farinae* extracts (Miles Inc, Spokane, Wash). The duration of immunotherapy ranged from 5 to 15 years, and the mean value was 8.9 years. All the patients satisfied the following criteria prior to re-

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SCORING OF NASAL SYMPTOMS

Sneezing	
0	No sneezing attacks
1	1-5 sneezing attacks
2	6-10 sneezing attacks
3	Over 11 sneezing attacks
Rhinorrhea	
0	No nose blowings
1	1-5 nose blowings
2	6-10 nose blowings
3	Over 11 nose blowings
Nasal obstruction	
0	No nasal obstruction
1	Nasal obstruction without mouth breathing
2	Nasal obstruction with sporadic mouth breathing
3	Nasal obstruction with predominant mouth breathing

Modified from Okuda et al.[11]

ceiving immunotherapy: typical symptoms of perennial allergic rhinitis, positive skin test for *D farinae*, positive nasal provocation test for *D farinae*, eosinophilia in nasal smears, and no previous immunotherapy. Thirty of the 48 patients had continuously undergone immunotherapy for 5 to 9 years, and the remaining 18 patients had continuously undergone immunotherapy for over 10 years.

Mode of Immunotherapy. The patients were given subcutaneous injections with Hollister-Stier standardized *D farinae* extracts (Miles Inc), with prior informed consent. The individual initiative dose was determined as indicated by the end point concentration determined by the threshold skin test. Doses were increased by 50% at weekly intervals until the individual maximum tolerated dose was reached. The maximum tolerated dose was 3,000 AU in 13 of 16 cases. The maximum tolerated dose was attained in 4 to 8 months with the once-weekly program. A weekly injection of this dose was administered for the first 3 to 5 months after attainment of the maintenance dose, and then the patients received injections of this dose on a biweekly basis for the next 3 to 5 months, followed by a monthly schedule. Finally, the interval between injections was gradually increased to up to 2 months in the absence of an episode of symptomatic relapse. After each injection, the patients were asked to remain under our supervision for a minimum of 30 minutes and to report any symptoms that they might have experienced. No concomitant medication affecting nasal symptoms was admitted throughout the period, with prior informed consent. All the patients chose to continue the treatment to avoid possible relapse even if they had no nasal symptoms.

percent decrease in symptomatic scores (%)

$$= \frac{\text{symptomatic scores before IT} - \text{symptomatic scores after IT}}{\text{symptomatic scores before IT}} \times 100$$

percent decrease in IgE antibodies (%)

$$= \frac{\text{IgE antibodies before IT} - \text{IgE antibodies after IT}}{\text{IgE antibodies before IT}} \times 100$$

percent increase in IgG4 antibodies (%)

$$= \frac{\text{IgG4 antibodies after IT} - \text{IgG4 antibodies before IT}}{\text{IgG4 antibodies before IT}} \times 100$$

Fig 1. Formulas. IT — immunotherapy.

Clinical Evaluation. The clinical evaluation of allergic rhinitis symptoms was performed twice in each patient: before the initiation of immunotherapy and on a variant time course of immunotherapy, according to the criteria of Okuda et al,[11] using daily symptom diary cards. All the patients were asked to complete daily symptom diary cards in the 14 days before each clinical assessment. In each patient, the second clinical assessment was performed in the same month that the first assessment was done before immunotherapy. The daily symptom card includes the number of sneezing attacks, the number of nose blowings, and the degree of nasal obstruction. A careful survey of the diary card graded the three nasal symptoms (sneezing, rhinorrhea, and obstruction) on a scale of 0 to 3, depending on severity (see Table[11]). The average daily scores of the sum total of the three nasal symptom scores were used as symptom scores (maximum: 9). The 48 patients were divided into two groups according to the clinical effectiveness evaluation based on the symptom scores after immunotherapy (marked response, 2 or less; moderate response, 3 or more). The percent decrease in symptom scores following immunotherapy was calculated from the formulas in Fig 1.

Determination of D farinae*–Specific IgE and IgG4 Antibodies.* Serum samples were collected twice at the time of clinical assessment from each patient. These serum samples were separated, stored at –60°C, and never retrieved prior to use for the determination of *D farinae*–specific IgE antibodies and IgG4 antibodies. All the serum samples were tested simultaneously. *Dermatophagoides farinae*–specific IgE antibodies in serum were determined by the Kallestad Allercoat System,[12] and the results were expressed in ARU per milliliter.

The IgG4 antibodies specific to *D farinae* in serum were determined by the enzyme-linked immunosorbent assay as previously described by Engvall and

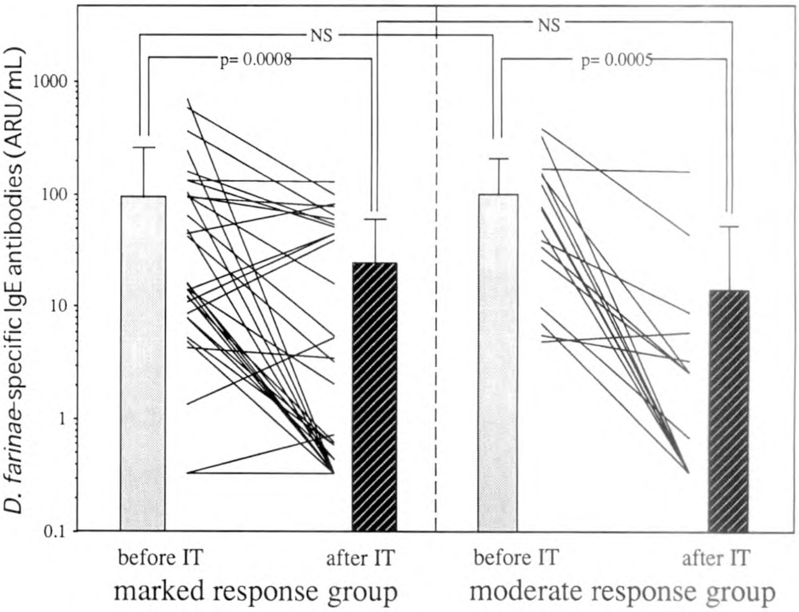

Fig 2. Changes in *Dermatophagoides farinae*–specific IgE antibodies in marked and moderate response groups. Level of IgE antibodies significantly decreased following immunotherapy in both groups. NS — not significant.

Perlman[13] with some modifications: the methodology and justification of the method employed in this study have been described elsewhere.[14] The percent decrease in IgE antibodies, as well as the percent increase in IgG4 antibodies following immunotherapy, was calculated from the formulas in Fig 1.

Statistical Analysis. Significant changes in the levels of *D farinae*–specific antibodies were statistically analyzed by means of the Wilcoxon signed-ranks test. Significant differences between the two different groups were statistically analyzed by means of the Mann-Whitney U-test. Spearman's correlation coefficient (rs) was calculated to examine a possible correlation between two different parameters, and a significant correlation was statistically analyzed by means of Spearman's rank correlation analysis. In any statistical analysis of this study, significance was also accepted when p was less than .05.

RESULTS

Clinical Evaluation. The pretreatment symptom scores of the 48 patients studied ranged from 5 to 9, with a mean of 7.21. Every patient demonstrated somewhat of a decrease in the symptom scores following immunotherapy. The symptom scores of the 48 patients after immunotherapy varied from 0 to 5, with a mean of 1.38. According to the clinical effectiveness evaluation based on the symptom scores after immunotherapy, the marked response group and the moderate response group consisted of 32 and 16 patients, respectively. The symptom scores (mean ± SD) prior to receiving immunotherapy were 7.34 ± 1.15 in the marked response group and 6.94 ± 1.06 in the moderate response group. There was no significant difference between the pretreatment symptom scores of the two groups. The symptom scores of the marked response group and the moderate response group after immunotherapy were 0.47 ± 0.62 and 3.19 ± 0.54, respectively. The mean of the percent decrease in symptom scores following immunotherapy was 93.73% ± 8.28% in the marked response group and 52.91% ± 11.77% in the moderate response group.

D farinae*–Specific IgE Antibody*. The levels of *D farinae*–specific IgE antibodies of the 48 patients studied were significantly decreased following immunotherapy ($p < .0001$; 99.74 ± 152.38 ARU/mL before immunotherapy and 21.77 ± 37.37 ARU/mL after immunotherapy). The rate of decrease in IgE antibodies of the 48 patients following immunotherapy was 36.61% ± 112.93%. However, 7 of the 48 patients demonstrated an elevation of IgE antibodies following immunotherapy, and 6 of the 7 patients nevertheless responded markedly to immunotherapy, according to our clinical evaluation criteria. The levels of *D farinae*–specific IgE antibodies before immunotherapy were 98.46 ± 170.11 ARU/mL in the marked response group and 102.30 ± 113.76 ARU/mL in the moderate response group. The pretreatment level of IgE antibodies was not significantly different between the two groups ($p = .304$). The levels of IgE antibodies after immunotherapy were 25.41 ± 36.65 ARU/mL in the marked response group and 14.51 ± 40.00 ARU/mL in the moderate response group. The IgE antibody level significantly decreased following immunotherapy in both groups (marked response group, $p = .0008$; moderate response group, $p = .0005$; Fig 2). The percent decrease of the mean values of IgE antibodies was 74.19% in

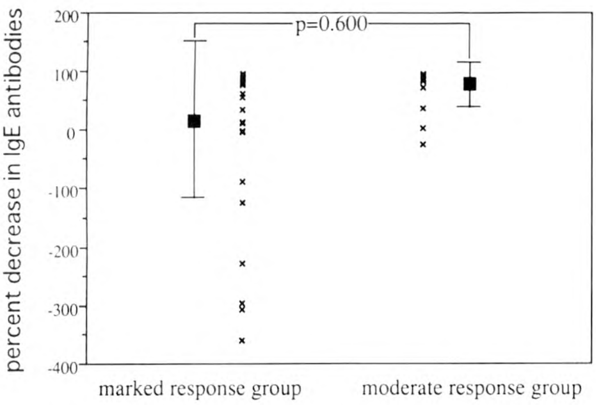

Fig 3. Percent decrease in *D farinae*–specific IgE antibodies following immunotherapy in marked and moderate response groups. Mean of individual percent decrease in IgE antibodies following immunotherapy is 18.55% ± 132.04% in marked response group and 78.73% ± 36.76% in moderate response group. There is no significant difference between rate of decrease in IgE antibodies in two groups.

the marked response group and 85.82% in the moderate response group. The level of the individual percent decrease in IgE antibodies following immunotherapy was not significantly different between the two groups (p = .600; marked response group, 18.55% ± 132.04%; moderate response group, 78.73% ± 36.76%; Fig 3).

D farinae–*Specific IgG4 Antibody*. The levels of *D farinae*–specific IgG4 antibodies of the 48 patients studied were significantly increased following immunotherapy ($p < .0001$; 0.66 ± 1.09 U/mL before immunotherapy and 5.63 ± 5.26 U/mL after immunotherapy). The rate of increase in IgG4 antibodies of the 48 patients following immunotherapy was 1,312.74% ± 1,453.98%. On the contrary, however, 2 patients demonstrated a decrease in IgG4 antibodies following immunotherapy, and both of them were included in the moderate response group. Each of the 7 patients who failed to decrease IgE antibodies showed a considerable elevation of IgG4 antibodies following immunotherapy. The levels of *D farinae*–specific IgG4 antibodies before immunotherapy were 0.70 ± 1.31 U/mL in the marked response group and 0.56 ± 0.38 U/mL in the moderate response group. There was no significant difference between the pretreatment levels of IgG4 antibodies of the two groups. The levels of IgG4 antibodies after immunotherapy were 7.69 ± 5.29 U/mL in the marked response group and 1.51 ± 1.28 U/mL in the moderate response group. The levels of IgG4 antibodies in the two groups following immunotherapy were significantly higher than the corresponding pretreatment levels (marked response group, $p < .0001$; moderate response group, $p = .0071$), but the level of IgG4 antibodies after immunotherapy in the marked response group was significantly higher than that in the moderate response group ($p < .0001$; Fig 4). In addition, the rate of increase in IgG4 antibody level of the marked response group (1,873.20% ± 1,485.44%) was significantly larger than that of the moderate response group (191.80% ± 231.31%; $p < .0001$; Fig 5).

Correlation Analysis. There was no significant correlation between the rate of decrease in symptom scores and the rate of decrease in IgE antibodies of the 48 patients studied ($r_s = -.221$; $p = .0720$; Fig 6). On the other hand, a significant positive correlation was observed between the rate of decrease in symptom scores and the rate of increase in IgG4 antibodies of the 48 patients studied following immunotherapy

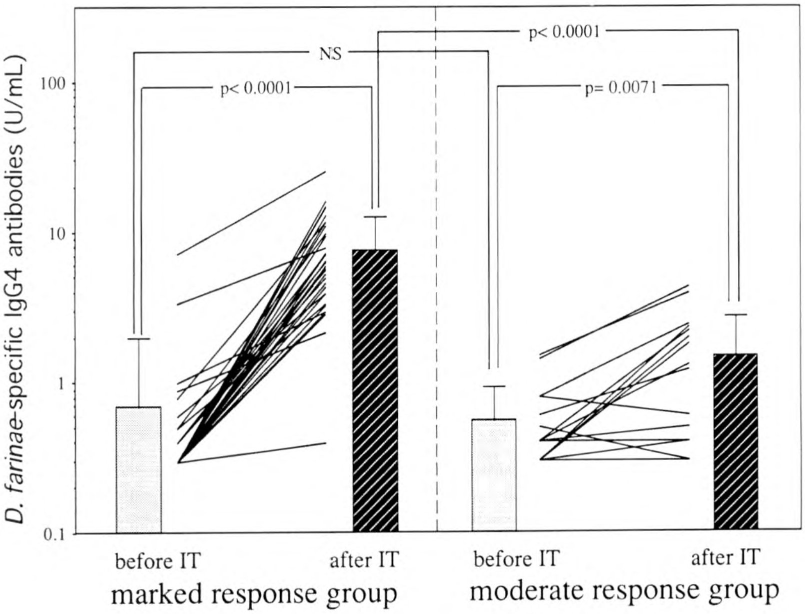

Fig 4. Changes in *D farinae*–specific IgG4 antibodies in marked and moderate response groups. Level of IgG4 antibodies significantly increased following immunotherapy in both groups. There is no significant difference between pretreatment levels of two groups. However, level of IgG4 antibodies in marked response group after immunotherapy is significantly higher than that in moderate response group.

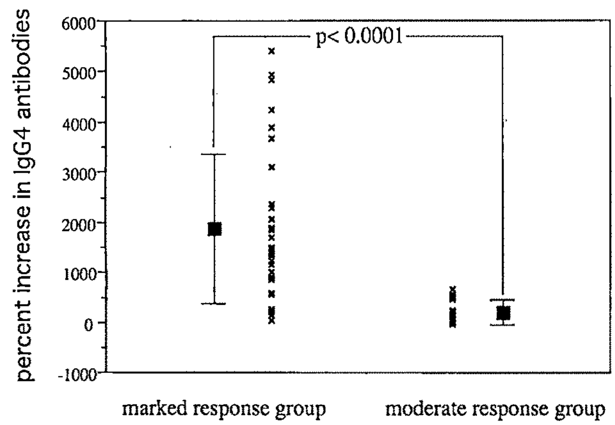

Fig 5. Percent increase in *D farinae*–specific IgG4 antibodies following immunotherapy in marked and moderate response groups. Mean of individual percent increase in IgG4 antibodies is 1,873.20% ± 1,485.44% in marked response group and 281.80% ± 420.71% in moderate response group. Significantly larger increase in IgG4 antibodies is observed in marked response group.

(r_s = .561; p = .0002; Fig 7).

DISCUSSION

Mechanisms for the clinical benefit of immunotherapy have been suggested, including reduction in serum specific IgE antibody level,[5] formation of blocking antibody,[7-9,14] decreased reactivity to allergen of target cells, and decreased number and sensitivity to allergen of basophils.[4,15-17]

It was previously postulated that immunotherapy could decrease IgE synthesis, but the review of the literature demonstrates that this reduction could not occur within a few years of immunotherapy.[5-7] Our previous study also failed to show any reduction in IgE antibody level even in patients with a good clinical outcome during the early phase of immunotherapy.[14,18] However, our present study has clearly demonstrated that the allergen-specific IgE antibody level significantly decreases after more than 5 years of immunotherapy. An initial increase followed by a decline of allergen-specific IgE antibodies may be caused by an early increased activation of IgE-producing B-cells followed by suppression.[19] Such changes in B-cell activity following long-term immunotherapy are probably regulated by the cytokine profile. According to prevalent concepts, these events might be induced by an effect of immunotherapy on T lymphocytes with alteration from a predominant Th2 response to a Th1 response, which results in reduction in interleukin-4 and altered regulation of IgE synthesis, thus leading to a decrease in allergen-specific IgE antibodies.[10,20]

A decrease in allergen-specific IgE antibodies could theoretically develop a good clinical course beyond controversy. Several lines of our results, however, have demonstrated that a decline of IgE antibodies is not always a key mechanism of clinically successful immunotherapy. First of all, there was no significant difference between the rate of decrease in IgE antibodies of the marked response group and the moderate response group, and moreover, the rate of decrease in IgE antibodies did not correlate with the decrease in symptom scores. In addition, 6 of 7 patients whose IgE antibodies were elevated following immunotherapy obtained marked clinical benefit. This overwhelming evidence argues against a critical role for a decline of IgE antibodies

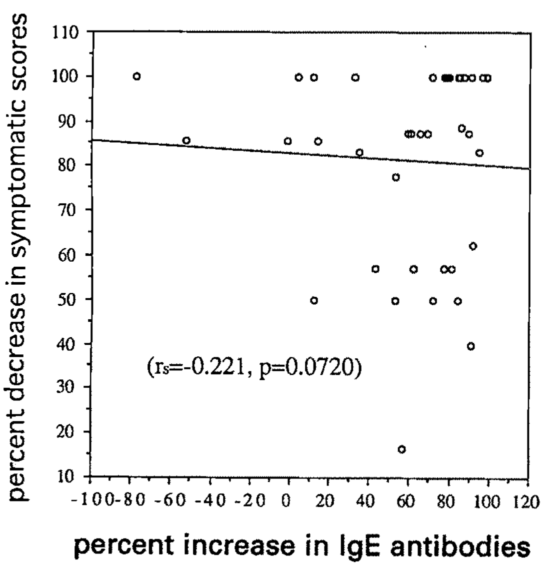

Fig 6. Percent decrease in *D farinae*–specific IgE antibodies versus rate of decrease in symptom scores. Percent decrease in IgE antibodies does not significantly correlate with percent decrease in symptom scores.

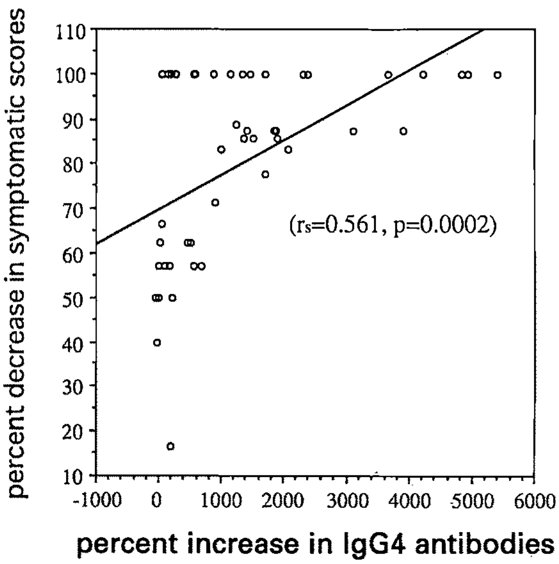

Fig 7. Percent increase in *D farinae*–specific IgG4 antibodies versus percent decrease in symptom scores. Percent increase in IgG4 antibodies significantly correlates with percent decrease in symptom scores.

in the major mechanisms of immunotherapy.

An experimental possibility has recently been presented that allergen-specific IgG4 antibodies might act as so-called blocking antibodies to inhibit IgE-mediated immunologic reactions.[21-23] Considerable attention has been devoted to the dynamics of IgG4 antibodies following immunotherapy during the last decade, and there is now general agreement that the IgG4 antibody level increases considerably during immunotherapy.[3,7-9] The role of IgG4 antibodies in the clinical efficacy of immunotherapy, however, remains controversial. It was previously advocated that a rise in IgG4 antibodies is strongly associated with treatment failure,[24] whereas this idea has not been supported by several other lines of investigation.[3,7-9] Most investigators are in favor of the doctrine that IgG4 antibodies play an important role as blocking antibodies in immunotherapy. However, critics are skeptical of a clinical role for IgG4 antibodies, advocating instead the idea that the increase in IgG4 antibodies is simply a marker of antigen exposure following subcutaneous injection and probably represents an epiphenomenon unrelated to the underlying working mechanism of clinically successful immunotherapy.[10,25]

To definitively establish the clinical significance of IgG4 antibodies, the statistical correlation should be analyzed between the clinical efficacy, especially symptom scores, and the percent increase in IgG4 antibodies. However, a clear correlation has yet to be established with symptom scores. In the opinion of the authors, immunotherapy retains a place in today's treatment of allergic rhinitis in that it has the capacity to completely alleviate nasal symptoms and possibly may have the potential for long-term cure after long-term active treatment, unlike conventional drug treatments. This study aimed at investigating the change in IgG4 antibodies induced by more than 5 years of immunotherapy and to examine the statistical correlation between IgG4 antibodies and symptom scores.

The percent increase in IgG4 antibodies was significantly larger in the marked response group than in the moderate response group. Also, a moderate correlation was statistically documented between the percent decrease in symptom scores and the percent increase in IgG4 antibodies. Taking both lines of evidence together, we consider that an elevation of IgG4 antibodies during immunotherapy makes a contribution to symptomatic relief as one of the working mechanisms in allergen-specific immunotherapy for perennial allergic rhinitis. However, our study included no untreated control group and examined IgG4 antibodies before and after 5 or more years of immunotherapy. Therefore, serial determination of IgG4 antibodies in immunotherapy patients, as well as untreated controls, is necessary to completely establish the clinical role of IgG4 antibodies in allergen-specific immunotherapy.

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RECONSTRUCTIVE MICROSURGERY IN HEAD AND NECK CANCER

The First International Symposium on Reconstructive Microsurgery in Head and Neck Cancer will be held in Pavia, Italy, September 11-13, 1997. For more information, contact M. Benazzo, MD, University of Pavia, Policlinico S. Matteo, P.le Golgi 2, 27100 Pavia, Italy; telephone +39 382 528184; fax +39 382 528184; e-mail: orl37@ipv36.unipv.it or mullacc@mbox.vol.it.

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FINE THREE-DIMENSIONAL STRUCTURE OF PERICYTES IN THE VOCAL FOLD MUCOSA

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KURUME, JAPAN

An investigation was carried out to determine the fine three-dimensional structure of pericytes in excised human vocal fold mucosa, by means of scanning and transmission electron microscopic observation. The results are summarized as follows. 1) There were many pericytes around the true capillaries, arterial capillaries, and venous capillaries in the vocal fold mucosa. 2) Newborns had pericytes around the capillaries in the vocal fold mucosa. 3) The pericytes had bulged fusiform or polygonal cell bodies and branching processes. The branching processes consisted of long and relatively thick longitudinal ones and short circumferential ones. 4) The cell body and processes of the pericytes encircled the capillaries, and the tips of the processes formed intercellular tight junctions with endothelial cells and made a firm connection with them. 5) The pericytes had many cytoplasmic filaments. 6) The pericytes in the vocal fold mucosa appeared to support and protect capillary walls in the vibrating tissue.

KEY WORDS — histology of larynx, larynx, pericyte, vocal folds.

INTRODUCTION

The structures of the blood vessels are unique at the vocal fold edge,[1-3] where only small vessels, including arterioles, venules, and capillaries, are present. The vessels enter the vocal fold edge from the anterior or posterior end of the membranous vocal fold and run essentially parallel to the edge. These structures of the vessels in the vocal fold mucosa are suited for vibration.[1-3]

Pericytes surround capillaries in the vocal fold mucosa.[4,5] Their fine structure and functions remain unclear. The fine three-dimensional structure of pericytes in the vocal fold mucosa was examined in this study by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Possible functions are proposed based on the results.

MATERIALS AND METHODS

Five normal human adult larynges, with ages ranging from 37 to 65 years (three males and two females), and three normal newborn larynges (all were term, one boy and two girls) obtained from autopsy cases were used. One vocal fold of each adult larynx was investigated by SEM, and the other, by TEM. Vocal folds of newborn larynges were investigated by TEM.

For SEM, each specimen was fixed in 2.5% glutaraldehyde at 4°C for 2 hours. After rinsing with cacodylate buffer solution, the collagen fibers were chemically digested by the modified sodium hydroxide (NaOH) maceration method.[6] All specimens were immersed in 6N NaOH for 10 minutes at 65°C, washed in physiological saline solution, and postfixed in 2% osmium tetroxide with Millonig's phosphate buffer solution[7] at 4°C for 2 hours. This was followed by dehydration in a graded series of concentrations of ethanol, immersion in tert-butylalcohol, and drying by the tert-butylalcohol freezing method. The specimens were then sputter-coated with gold and examined under a Hitachi S-800 scanning electron microscope.

For TEM, each specimen was fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution, and postfixed in 2% osmium tetroxide with Millonig's phosphate buffer solution[7] at 4°C for 2 hours. They were then dehydrated in a graded series of concentrations of ethanol and embedded in epoxy resin. Semithin sections were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined with a light microscope. Thin sections were made with an ultramicrotome and stained with uranyl acetate and lead citrate. The sections were then observed under a JEM-2000EX transmission electron microscope.

RESULTS

Scanning Electron Microscopy. Figure 1 shows SEM findings of pericytes in the vocal fold mucosa. Collagenous fibers were chemically digested, and elastic fibers and cells alone remained following treatment by the modified NaOH maceration method.

There were only small vessels, including arteri-

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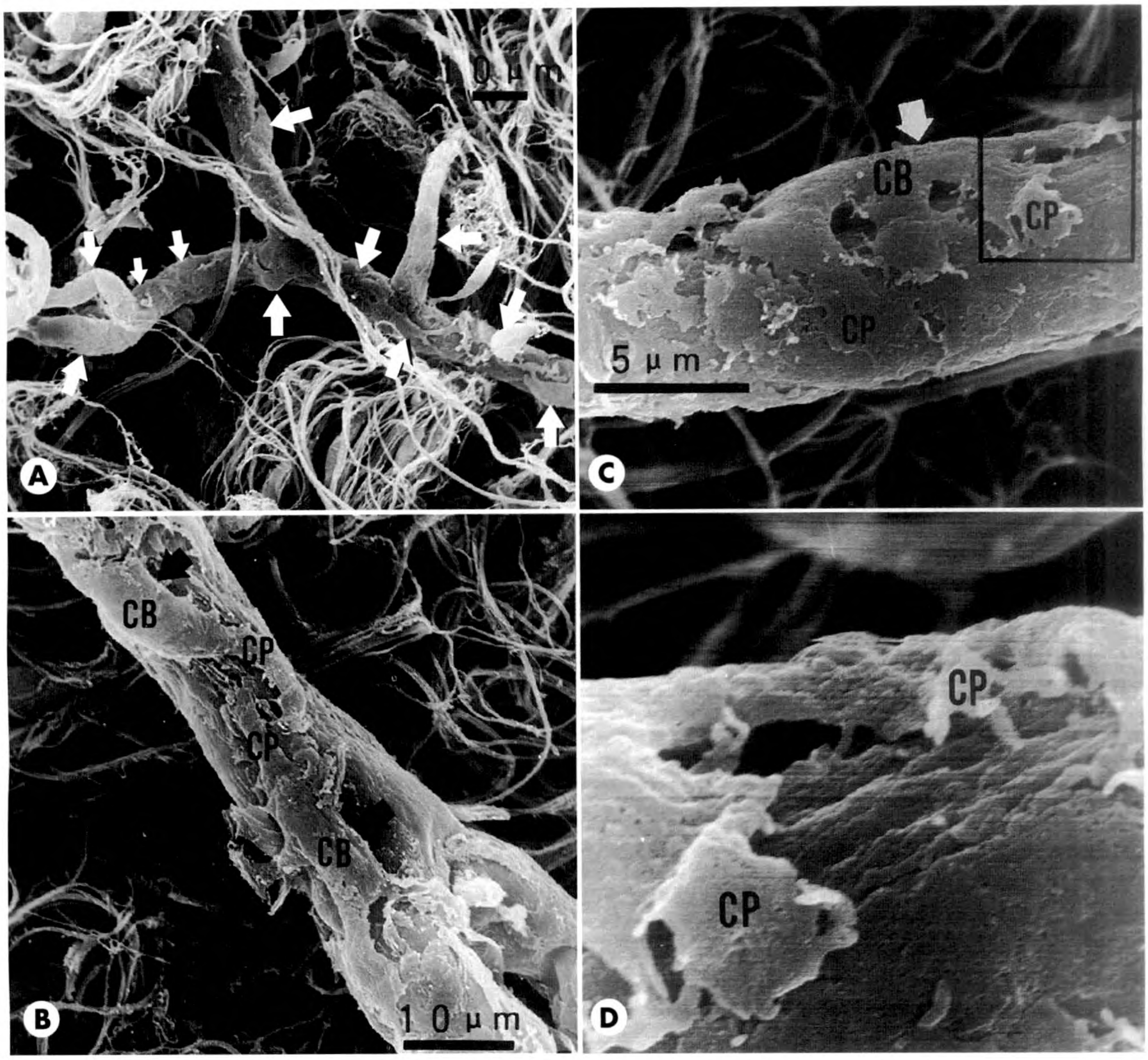

Fig 1. Scanning electron microscopy of pericytes (arrows) in vocal fold mucosa. A) Shows capillaries. B,C) CB — cell body, CP — cytoplasmic processes. D) Enframed area from C.

oles, venules, and capillaries, in the vocal fold mucosa, running roughly parallel to the vocal fold edge.

Many pericytes could be seen around the capillaries in the vocal fold mucosa (Fig 1A). Each pericyte had a cell body and branching cytoplasmic processes (Fig 1B). The cell bodies were bulged fusiform or polygonal. Branching cytoplasmic processes consisted of long and relatively thick longitudinal ones that were parallel to the axis of the vessel and short circumferential ones (Fig 1B). The pericytes were 5 to 10 μm by 15 to 30 μm and cell bodies were 5 to 10 μm by 10 to 15 μm in size. The cell bodies of the pericyte attached to capillary endothelial cells (Fig 1C), and the branching processes attached to the capillary endothelial cells at the tips (Fig 1D) and encircled the capillaries (Fig 1C). The processes of the pericytes were fingerlike or clawlike in appearance and, as well as the cell bodies, appeared to grasp the vessels.

Figure 2A shows SEM findings of arterioles in the vocal fold mucosa. Smooth muscle cells in the arterioles completely encircled the vessels. Arterial capillaries, the area of transition from an arteriole to a capillary, were intermediate in form from smooth muscle cells to pericytes (Fig 2B). Figure 2C shows a venous capillary, the area of transition from a capillary to a venule in the vocal fold mucosa. A surface view also appeared intermediate in form from smooth muscle cells to pericytes.

Transmission Electron Microscopy. Figure 3A shows TEM findings on pericytes. The cell body and processes of the pericyte encircled the capillary. The pericyte contained a large nucleus. Components in the cytoplasm such as rough endoplasmic reticulum, mitochondria, and free ribosomes were present. Many cytoplasmic filaments could be seen, not only in cell bodies, but in processes as well (Fig 3B). The filaments came together to form dense bodies (Fig

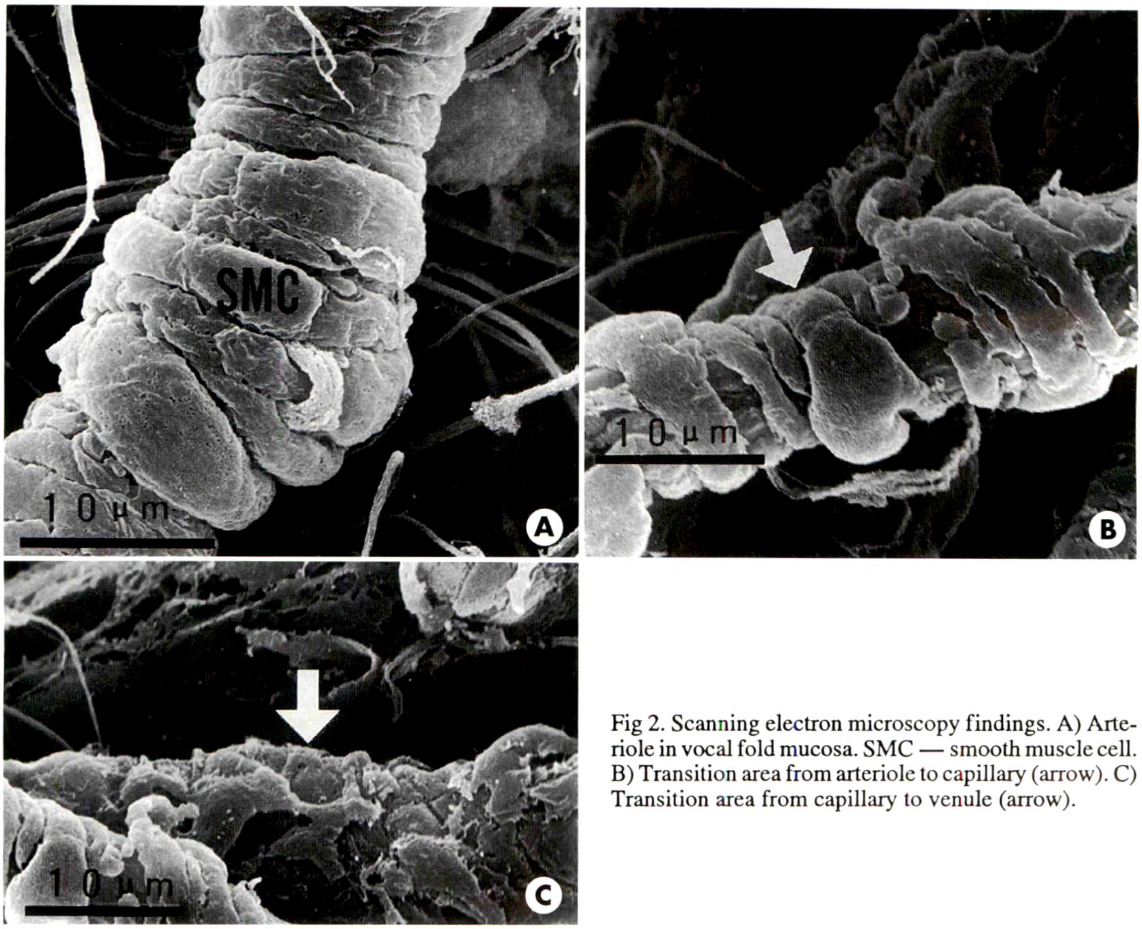

Fig 2. Scanning electron microscopy findings. A) Arteriole in vocal fold mucosa. SMC — smooth muscle cell. B) Transition area from arteriole to capillary (arrow). C) Transition area from capillary to venule (arrow).

3B). Adjacent to the endothelial cells, a dense meshwork of cytoplasmic filaments in the processes was noted. On the outside of the processes, many pinocytotic vesicles were observed. Cell bodies and processes encircled the capillaries and attached to their walls (Fig 3A,C). The cell bodies and endothelial cells remained separated by a gap of 300 to 500 nm (Fig 3A,C). The processes were in close contact with endothelial cells, sharing a common basement membrane with endothelial cells (Fig 3D). The tips of the processes formed intercellular tight junctions with endothelial cells (Fig 3D). These regions were considered to correspond to areas in Fig 1D showing SEM findings.

Figure 3E shows TEM findings on pericytes of the newborn. The cell body and processes of the pericytes encircled the capillaries in the vocal fold mucosa at birth, just as in the adult larynges.

DISCUSSION

Zimmermann[8] studied the capillary pericytes by silver staining using light microscopy. Electron microscopic studies have been conducted on capillary pericytes in other organs.[9-14] The number and shape of capillary pericytes differ according to the organs and tissues.[9-14] The number of capillary pericytes is related to the density of the capillary bed.[13] Their shape and distribution may possibly be related to organ function.

The functions of pericytes still remain unclear. Synthesis, mechanical support, protection, detection, differentiation, and capillary contraction have been suggested.[14] Cytoplasmic filaments were previously noted in pericytes and thus they were considered contractile cells that modulate microvascular blood flow.[10,11]

The vocal fold is a vibrating structure, the most important vibrating tissue of which is the lamina propria, particularly its superficial layer.[1] The blood vessels are unique at the vocal fold edge,[1-3] where only small vessels such as arterioles, venules, and capillaries are present.[1-3] These vessels enter the vocal fold edge from the anterior or posterior end of the membranous vocal fold and run roughly parallel to the edge.[1-3] Blood vessels in the mucosa at the free edge are clearly distinct from those in the mucosa of the upper and lower surfaces of the vocal fold, as well as those in the vocalis muscle.[3] The structures of the vessels in the vocal fold mucosa are suited for vibration.[1-3]

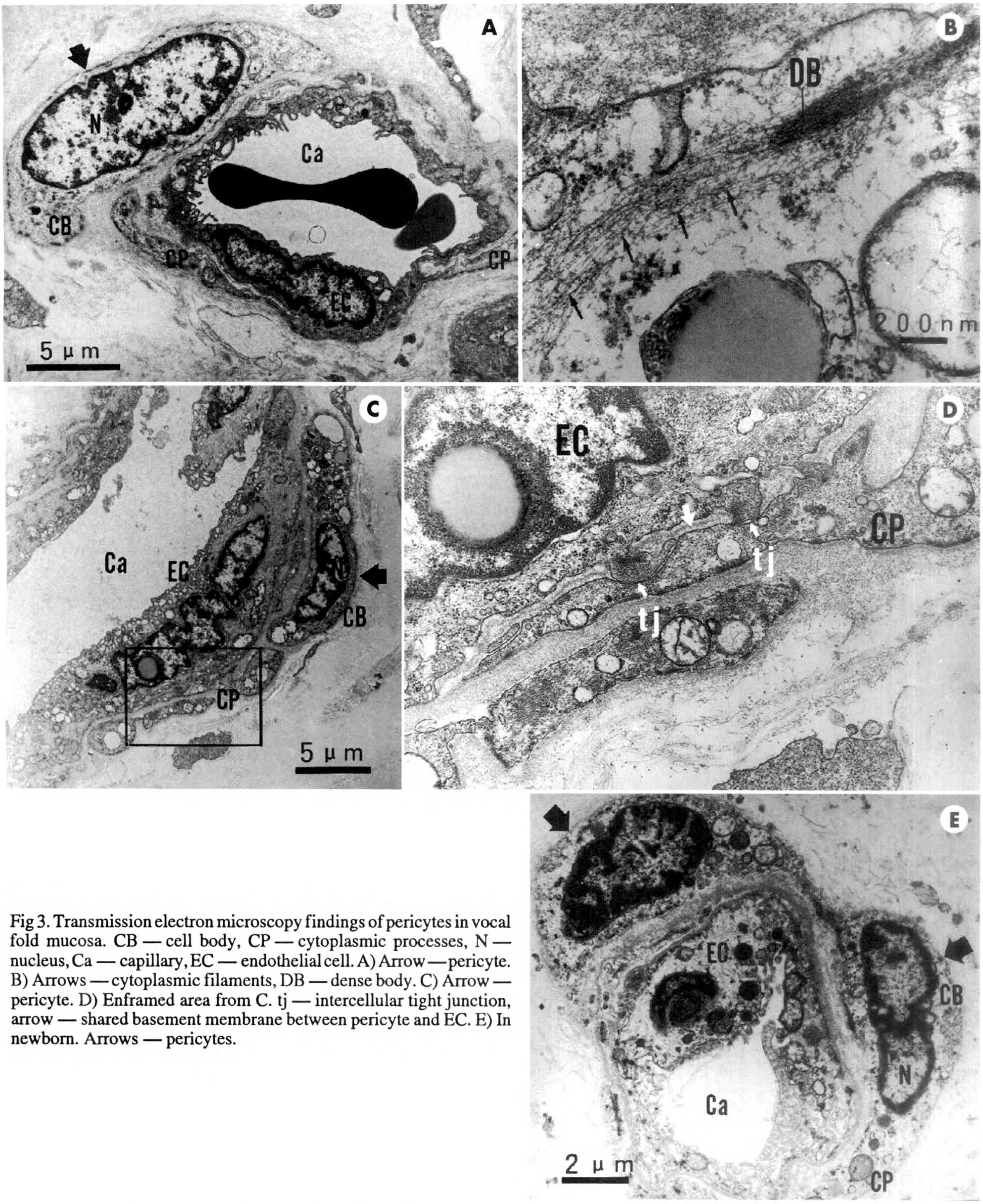

Fig 3. Transmission electron microscopy findings of pericytes in vocal fold mucosa. CB — cell body, CP — cytoplasmic processes, N — nucleus, Ca — capillary, EC — endothelial cell. A) Arrow — pericyte. B) Arrows — cytoplasmic filaments, DB — dense body. C) Arrow — pericyte. D) Enframed area from C. tj — intercellular tight junction, arrow — shared basement membrane between pericyte and EC. E) In newborn. Arrows — pericytes.

Pericytes were previously noted around capillaries in the vocal fold mucosa.[4,5] In this study, the fine three-dimensional structure of pericytes in the vocal fold mucosa was studied by SEM and TEM. The pericytes were found to consist of a cell body and branching processes. Cytoplasmic filaments were present in the cytoplasm. The pericytes in the vocal fold mucosa were essentially the same as those in other organs.[9-14] Many pericytes could be seen around capillaries, arterial capillaries, and venous capillaries in the vocal fold mucosa. The most characteristic finding of the pericytes in the vocal fold mucosa was the presence of processes thicker than those in other organs. Pericytes encircled the capillary walls. The tips of the processes formed tight intercellular junctions with endothelial cells. The cell bodies and processes appeared to grasp the vessels, and support and protect capillary walls. Their thickness and firm connection with endothelial cells render them particularly suitable for such support and protection. Many cytoplasmic filaments could be seen to come together to form dense bodies. The pericytes thus provide great support and protection for the capillary walls. The pericytes encircled the capillaries in the vocal fold mucosa of the newborn larynges and appeared ready to provide support and protection of the vessels after birth. The vessels in the vocal fold mucosa, which is the vibrating portion of the vocal folds, do not rupture easily, even during frequent and strong vibrations. The blood flow of the vocal fold mucosa is reduced during phonation,[15] but increases thereafter. The pericytes in the vocal fold mucosa appear to provide mechanical support and protection to the capillary walls, particularly during phonation.

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PRESSURE GRADIENTS AFFECTING THE LABYRINTH DURING HYPOBARIC PRESSURE

EXPERIMENTAL STUDY

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Hypobaric effects on the perilymph pressure were investigated in 18 cats. The perilymph, tympanic cavity, cerebrospinal fluid, and systemic and ambient pressure changes were continuously recorded relative to the atmospheric pressure. The pressure equilibration of the eustachian tube and the cochlear aqueduct was studied, as well as the effects of blocking these channels. During ascent, the physiologic opening of the eustachian tube reduced the pressure gradients across the tympanic membrane. The patent cochlear aqueduct equilibrated perilymph pressure to cerebrospinal fluid compartment levels with a considerable pressure gradient across the oval and round windows. With the aqueduct blocked, the pressure decrease within the labyrinth and tympanic cavities was limited, resulting in large pressure gradients toward the chamber and the cerebrospinal fluid compartments, respectively. We conclude that closed cavities with limited pressure release capacities are the cause of the pressure gradients. The strain exerted by these pressure gradients is potentially harmful to the ear.

KEY WORDS — barotrauma, cat, cochlea, cochlear aqueduct, eustachian tube, hydrostatic pressure, intracranial pressure, labyrinth, middle ear, round window, tympanic membrane.

INTRODUCTION

In our previous studies on perilymph pressure during hypobaric pressure exposures,[1,2] we investigated the importance of the rate, magnitude, and duration of the pressure changes relative to the altered chamber pressure (PCh). The influence of ambient pressure changes on cochlear and vestibular function has been indicated in several reports of aerospace and underwater medicine,[3-6] as well as in studies on the effects of pressure chamber treatment of Meniere's disease.[7-10]

In this study, we present new results together with material from previous studies in order to appreciate which is of greater importance during exposure to hypobaric pressure: the large pressure deflections or the emerging pressure gradients across the membranes and channels of the skull.

The mechanical properties of the membranous labyrinth have been studied in order to evaluate the strength of the membranes.[11,12] The cause and consequences of perilymph fistulas with leakage to the tympanic cavity are still debated.[13-15] Studies have revealed daily variations of the cerebrospinal fluid pressure under normobaric conditions.[16] To date, research regarding the points and duration of the strain exerted on the inner ear structures during hypobaric exposure has not been presented.

The primary aim of this study is to investigate the perilymph pressure (PPl) changes and the pressure release effects of the cochlear aqueduct and eustachian tube in cats exposed to hypobaric pressure. We focus on the emergence of the pressure gradients between the middle and inner ears, as well as their surroundings: the chamber and the cerebrospinal fluid compartment. In this study, the hydrostatic pressure changes are indicated in relation to the atmospheric pressure.

MATERIAL AND METHODS

Eighteen healthy cats weighing 2.5 to 3.5 kg were used. The animals were intravenously anesthetized with ketamine hydrochloride or phenobarbital sodium. The ventilation was controlled with a respira-

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From the Department of Otolaryngology, University Hospital, Lund (Konrádsson), and the Department of Otolaryngology, University Hospital, Malmö (Carlborg), Sweden, and the Division of Otolaryngology, Duke University Medical Center, Durham, North Carolina (Farmer). This study was supported by grants from the Medical Faculty of the University of Lund, the Helga Hjärpstedt Foundation, the Swedish Federation of Hard of Hearing People (HRF), the Silent School Foundation, Fogarty International Research Fellowships, No. 1F05TW0350-01 IARG, and the National Institutes of Health, grant HL 07896. This study was done in part in 1983 in accordance with the guidelines of Duke University, which has been accredited by the American Association for Accreditation of Laboratory Animal Care since December 1975. This accreditation was and is in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*, published by the US Department of Health and Human Services. The parts of the study done in Sweden were in accordance with the Swedish government's guidelines for the use of laboratory animals.

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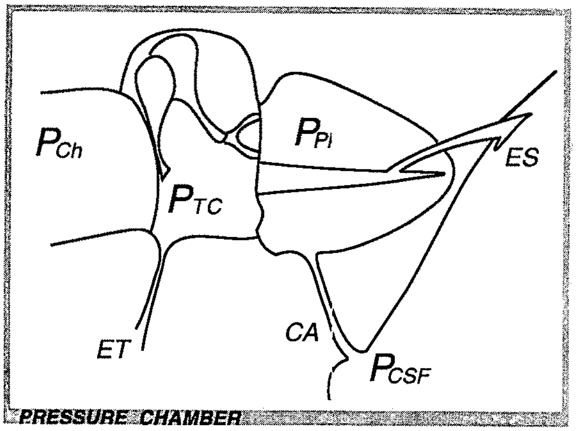

Fig 1. Schematic drawing of ear indicating chamber pressure (PCh), tympanic cavity pressure (PTC), perilymph pressure (PPl), cerebrospinal fluid pressure (PCSF), eustachian tube (ET), cochlear aqueduct (CA), and endolymphatic sac (ES).

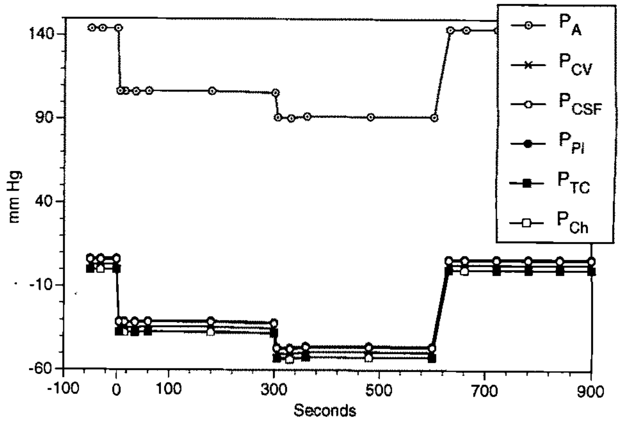

Fig 2. Change in chamber (PCh), tympanic cavity (PTC), perilymph (PPl), cerebrospinal fluid (PCSF), central venous (PCV), and arterial (PA) pressures relative to atmospheric pressure. Tympanic bulla is open. Data are from 1 representative animal. See text.

tor. Arterial oxygen saturation and acid-base levels were kept within normal limits. The pressure was continuously recorded from the tympanic cavity through the tympanic bulla (PTC), from the cerebrospinal fluid through the lateral ventricle of the brain (PCSF), from the internal jugular vein (PV), and from the femoral artery (PA). The PPl was measured in the vestibule by means of an extra-aural surgical approach to the labyrinth. This approach has been previously described,[17] and maintains the ear canal and the tympanic cavity intact. To exclude the physiologic opening of the eustachian tube, the tube was obstructed in some animals. A perforation (5 mm) of the tympanic bulla was made in all animals. In 9 animals, this opening was used to obstruct the cochlear aqueduct with dental amalgam. The perforation of the bulla was sealed with a rubber stopper containing two metallic cannulae (1 mm): one cannula for measuring the PTC and the other for manual pressure equilibration[2] (Fig 1).

The PCh was recorded with a differential capacitive pressure transducer. All other pressures — PPl, PTC, PCSF, PV, and PA — were recorded with Millar microtip pressure transducers PC 350, 5F (Miller Instruments, Inc, Houston, Tex). All the hydrostatic pressures reported here are relative to the prevailing atmospheric pressure (mean pressure 745 mm Hg), approximately 135 m above sea level.

Eighteen cats were studied during five different experimental conditions (1 through 5). In condition 1 (6 cats), the tympanic cavity was always open, but the cochlear aqueduct was either patent (4 cats) or blocked (2 cats). In conditions 2 through 5, the tympanic cavity was always sealed. The Table outlines the numbers of animals and experiments, as well as the status of the cochlear aqueduct and the eustachian tube patency.

HYPOBARIC PRESSURE

During conditions 1, 2, and 4, the manually controlled chamber pressure was reduced by approximately −40 mm Hg relative to the prevailing atmospheric pressure. The pressure level was then kept stable for 5 minutes, after which it was further reduced to approximately −52 mm Hg. After a stable level was maintained for 5 more minutes, the PCh was equilibrated to the atmospheric pressure. During condition 5, the chamber pressure was lowered (range −25 to −55 mm Hg) and kept stable for 2 to 4 minutes and then equilibrated to the atmospheric pressure. The experiments were concluded by an observation period of 5 minutes. During condition 3, the chamber

EXPERIMENTAL CONDITIONS OF TYMPANIC CAVITY, EUSTACHIAN TUBE, AND COCHLEAR AQUEDUCT, CHAMBER PRESSURE, AND NUMBERS OF CATS AND OF EXPERIMENTS CONDUCTED

Conditions	*Tympanic Cavity*	*Eustachian Tube*	*Cochlear Aqueduct*	*Pressure (mm Hg)*	*Cats/ Experiments*
1A	Open		Intact	−40	4/4
1B	Open		Blocked	−25 to −40	2/2
2	Closed	Blocked	Intact	−40	7/32
3	Closed	Intact	Intact	−25 to −55	4/24
4	Closed	Blocked	Blocked	−40	5/10
5	Closed	Intact	Blocked	−25 to −55	3/7

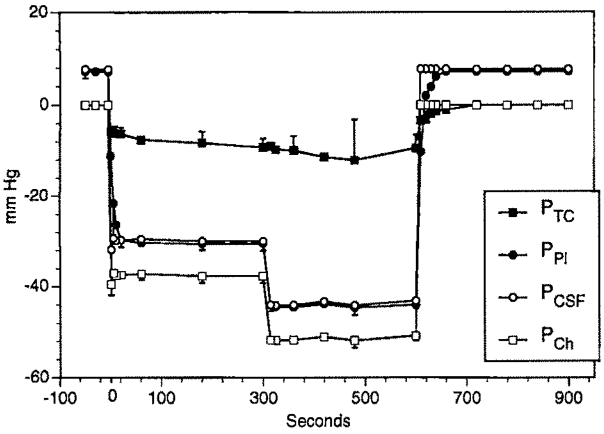

Fig 3. Change in chamber (PCh), tympanic cavity (PTC), perilymph (PPl), and cerebrospinal fluid (PCSF) pressures relative to atmospheric pressure. Eustachian tube is blocked, cochlear aqueduct open. Mean and SD values are based on 32 experiments on 7 animals.

pressure was lowered stepwise to the range of –25 to –55 mm Hg and equilibrated to atmospheric pressure after 4 minutes.

The pressure changes were induced at rates between 3.0 and 13.3 mm Hg/s. The experiments were repeated for each animal. After completion of the experiments, the system was tested for leakage. A mercury manometer was used to apply overpressure directly to the middle ear (range 0 to 60 mm Hg). The round and oval windows were microscopically examined.

STATISTICAL CALCULATIONS

The results are expressed as mean values ± SD. Student's *t*-test and the Wilcoxon signed rank sum tests were used as specified, and $p < .05$ was considered statistically significant.

RESULTS

CONDITION 1: TYMPANIC BULLA OPEN (FIG 2)

The effect of hypobaric pressure on the bodily pressures was studied. An open communication to the tympanic bulla was maintained during the experiments to eliminate the effect of the closed tympanic cavity. This prevented any pressure gradient between the chamber and the cavity. In 4 experiments on 4 cats, the cochlear aqueduct was physiologically patent, and in 2 experiments on an equal number of cats, the aqueduct was physiologically blocked.

During hypobaric pressure and the observation period of 5 minutes, all the pressures changed parallel to the PCh, and no changes in preexperimental pressure gradients were recorded. This was true regardless of the patency of the cochlear aqueduct.

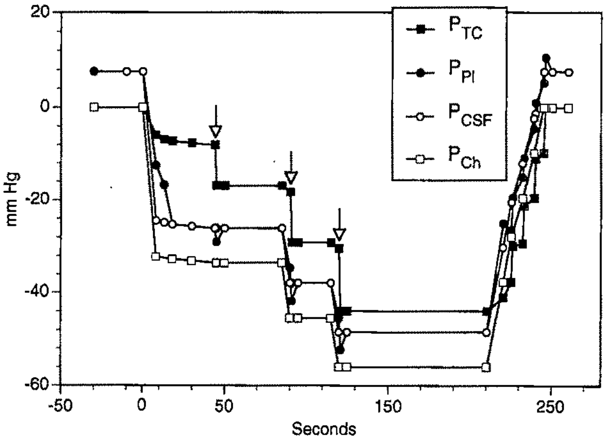

Fig 4. Change in chamber (PCh), tympanic cavity (PTC), perilymph (PPl), and cerebrospinal fluid (PCSF) pressures relative to atmospheric pressure. Eustachian tube and cochlear aqueduct are patent. Arrows indicate repeated physiologic openings of eustachian tube. Because of variable individual onset of tubal openings, data from 1 representative animal are shown.

CONDITION 2: EUSTACHIAN TUBE OBSTRUCTED AND COCHLEAR AQUEDUCT PATENT (FIG 3)

Tympanic Cavity Pressure Changes. The tympanic cavity was physiologically intact. Thirty-two experiments were performed on 7 cats.

The rate of PCh change was 7.7 ± 1.7 mm Hg/s, and the initial reduction of the PCh was to a level of –39.5 ± 2.4 mm Hg. The simultaneous change of the PTC was restricted, and a fall to a level of –5.9 ± 1.8 mm Hg was recorded. During the remainder of the first 5-minute period, the PTC was further reduced by 3.4 mm Hg.

When the PCh was reduced to a level of –52.0 ± 0.9 mm Hg, the initial effect on the PTC was slight. However, a slow, continuous, but limited fall of the PTC was recorded despite the stable PCh. When atmospheric pressure was restored in the chamber, the PTC level quickly rose to –3.4 ± 0.6 mm Hg, after which a slow approach to preexperimental levels was seen.

Labyrinthine Pressure Changes. The preexperimental levels of the PPl and the PCSF were 7.2 ± 1.8 and 7.7 ± 3.9 mm Hg, respectively. The initial fall of PPl was delayed, but within 30 seconds the PPl did reach the PCSF level. Similar pressure changes were observed during the second reduction of the PCh. As the PCh was normalized, a delay of the PPl pressure change was again recorded during adaptation to preexperimental levels.

CONDITION 3: EUSTACHIAN TUBE AND COCHLEAR AQUEDUCT PATENT (FIG 4)

Tympanic Cavity Pressure Changes. Four cats were exposed to hypobaric pressure changes in 24

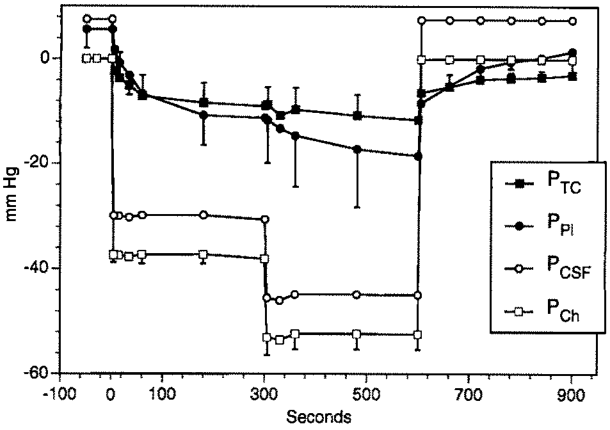

Fig 5. Change in chamber (PCh), tympanic cavity (PTC), perilymph (PPl), and cerebrospinal fluid (PCSF) pressures relative to atmospheric pressure. Eustachian tube and cochlear aqueduct are blocked. Mean and SD values are based on 10 experiments on 5 animals.

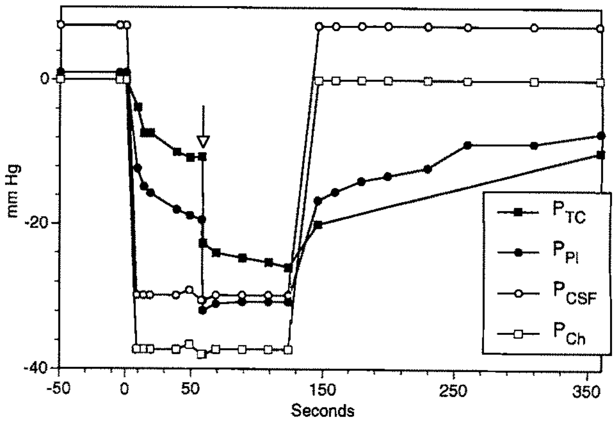

Fig 6. Change in chamber (PCh), tympanic cavity (PTC), perilymph (PPl), and cerebrospinal fluid (PCSF) pressures relative to atmospheric pressure. Eustachian tube is patent, cochlear aqueduct blocked. Arrow indicates physiologic opening of eustachian tube. Because of variable individual onset of tubal openings, data from 1 representative animal are shown.

experiments. The initial pressure changes were similar to those observed with the tube blocked, indicating no immediate leakage through the eustachian tube. In all experiments, the physiologic opening of the tube occurred spontaneously within 1 minute, resulting in a partial pressure equilibration between the PTC and the PCh. There was an individual variation of the time of tubal opening, as well as of the pressure levels at which the tube opened and closed. Consequently, the mean values, including pressure gradients, could not be statistically analyzed and compared as in the better-controlled conditions with the eustachian tube blocked.

However, the pressure changes were comparable in all the experiments conducted. Figure 4 shows the pressure changes recorded from a representative animal when the PCh was reduced to a level of –56.4 mm Hg in three steps. Repeated openings of the eustachian tube brought the PTC successively down to the level of –44.4 mm Hg, effectively reducing the pressure gradient toward the chamber.

Labyrinthine Pressure Changes. The preexperimental levels of the PPl and the PCSF were 5.7 ± 1.9 and 4.8 ± 3.5 mm Hg, respectively. The initial changes of the PCSF and the PPl were similar to those observed in experiments with the eustachian tube obstructed and the cochlear aqueduct patent. In the experiment shown (Fig 4), the first physiologic opening of the tube caused a significant but transient decrease of the PPl 3.8 mm Hg below the PCSF — a difference rapidly equilibrated. This phenomenon was observed in all experiments and also during the second and third lowerings of the PCh. When the PCh was slowly normalized, the PTC was simultaneously equilibrated to the PCh by repeatedly opening a valve attached to the cat's bulla. As before, the PCSF closely followed the PCh changes. The PPl returned to preexperimental values following the PCSF changes, exhibiting some deflections during PTC equilibration.

CONDITION 4: EUSTACHIAN TUBE AND COCHLEAR AQUEDUCT SURGICALLY BLOCKED (FIG 5)

Tympanic Cavity Pressure Changes. Ten experiments were conducted on 5 cats. The PCh was reduced to a level of –37.4 ± 1.4 mm Hg at the rate of 5.1 ± 1.7 mm Hg/s. The PTC changes induced were similar to those seen in the experiments described above. During the first minute, the PTC rapidly fell to a level of –7.1 ± 4.0 mm Hg, equaling 60% of the total pressure reduction. Despite a stable PCh, the PTC fall continued during the entire experiment, and the remaining 40% of the pressure reduction was recorded during the last 9 minutes.

When the PCh was equilibrated to atmospheric pressure, the PTC slowly approached, but did not reach, preexperimental levels during the observation period.

Labyrinthine Pressure Changes. The preexperimental levels of the PPl and the PCSF were 5.6 ± 3.6 and 6.6 ± 1.9 mm Hg, respectively. Compared to experiments with the cochlear aqueduct intact, the fall of the PPl was limited, but 50% of the total PPl reduction was recorded during the first minute of the experiment. The fall continued although the PCh was kept stable. The PPl fall was, in every experiment, larger than the corresponding PTC decline. A Wilcoxon signed rank test confirmed a significant ($p < .05$) difference already after 35 seconds of hypobaric pressure. After equilibration to atmospheric pressure, the PPl gradually normalized, but did not reach preexperimental levels during the observation period of 5 minutes.

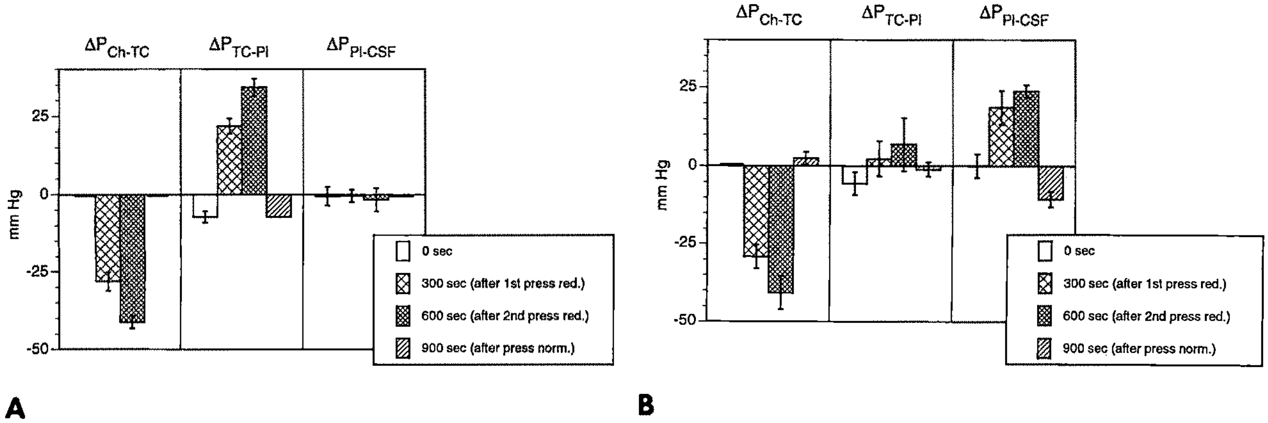

Fig 7. Pressure (press) gradients between chamber and tympanic cavity (ΔPCh-TC), tympanic cavity and perilymph (ΔPTC–PI), and perilymph and cerebrospinal fluid (ΔPPI-CSF) before pressure reductions (red.), during first (at 300 seconds) and second (at 600 seconds) pressure reductions, and after normalization (norm.) of chamber pressure. Mean values and 1 SD. A) See Fig 3 for reference. Eustachian tube is blocked and cochlear aqueduct patent (condition 2). B) See Fig 5 for reference. Eustachian tube and cochlear aqueduct are blocked (condition 4).

CONDITION 5: EUSTACHIAN TUBE PATENT AND COCHLEAR AQUEDUCT OBSTRUCTED (FIG 6)

Tympanic Cavity Pressure Changes. Three animals in 7 experiments were exposed to hypobaric pressure (range –37.6 to –39.1 mm Hg). Initially, the changes were similar to those observed with the eustachian tube and the cochlear aqueduct blocked. However, in all the experiments the tube was forced open within 2 minutes, but the timing, as well as the opening and closing pressure levels, varied individually. The pressure changes recorded during 1 representative experiment are shown in Fig 6. In this experiment, the partial pressure equilibration (arrow) lowered the PTC by 12 mm Hg, bringing it closer to the PCh level. At the end of each experiment, when the PCh was normalized, the PTC approached, but did not reach, preexperimental levels during the observation period.

Labyrinthine Pressure Changes. The initial change of the PPI was limited and not significantly different from that seen in experiments with the eustachian tube and the cochlear aqueduct obstructed. The partial pressure equilibration of the PTC caused a definite fall of the PPI (range 6.0 to 15.0 mm Hg). When the PCh was normalized, the PPI slowly approached, but did not reach, preexperimental levels during the observation period.

Manometric tests and microscopic examination performed after the experiments revealed no indications of leakage around the cannulae, nor signs of damage to the tympanic membrane or to the oval or round window membranes.

DISCUSSION

In this study, we focus on the changes in pressure and the emergence of pressure gradients between the chamber, tympanic cavity, perilymph, and cerebrospinal fluid, respectively, during hypobaric pressure exposures of healthy adult cats. The experimental conditions in this study are partly known from our previous publications on hypobaric pressure,[1,2] but here we present the results as values relative to the prevailing atmospheric pressure only.

The first experiments (condition 1) clearly demonstrate the influence of the physiologically intact tympanic cavity. With the tympanic bulla opened, no new pressure gradients toward the labyrinth emerge, as all the pressures recorded changed parallel during hypobaric pressure. This was independent of the patency of the cochlear aqueduct. The strict correlation of the bodily pressures to the ambient pressure changes can be explained as a result of the pressure effect on the body surface. The pressure changes are directly transferred to the body fluids and tissues, and correspondingly, the PV, PA, and PCSF react momentarily. These immediate and parallel changes of the PV, PA, and PCSF to the ambient PCh were seen in all the experiments conducted. When the tympanic cavity is open (condition 1, Fig 2), there is no buildup of new pressure gradients toward the labyrinth, nor any influence on the pressure relation between the labyrinth and the cerebrospinal fluid.

By closing the tympanic cavity and blocking the eustachian tube (condition 2, Fig 3), the pressure equilibration between the cavity and the chamber is prevented. The tympanic cavity now acts as a closed cavity, resisting external pressure changes. The limited PTC fall causes a considerable pressure gradient toward the surroundings, both the chamber and the labyrinth. This new pressure gradient momentarily affects the labyrinthine fluid pressure through the oval and particularly the round window,[18] preventing the immediate pressure adaptation to the ambient PCh change. This is seen as a brief delay in PPI equilibration through the intact cochlear aqueduct to the cerebrospinal fluid. This new pressure gradient between the labyrinth and the cerebrospinal fluid (ΔPPI–CSF) is transient. The duration, as well as the magnitude, of the pressure gradient varies individually due to the physiological patency of the aqueduct, the magnitude of the PTC gradient, and the rate of pressure change in the chamber. In this study, as in previous ones,[1,2] we observe that the pressure gradient is large at high rates, and small at slow rates, since the gradients caused by slower changes are effectively neutralized, and any lasting effect of the faster changes is prevented by the patent aqueduct — the typical characteristics of a low-pass filter. Consequently, a large and long-standing pressure gradient emerges between the labyrinth and the tympanic cavity (ΔPTC–PI) during the experiments with the cochlear aqueduct patent, whereas the ΔPPI–CSF is only transient (Fig 7A).

During the next set of experiments (condition 3, Fig 4), the eustachian tube and cochlear aqueduct were kept physiologically intact — a situation common in cats as well as in younger humans.[19,20] The individually varying opening time and opening and closing pressure levels of the eustachian tube make it difficult to calculate mean values regarding the pressure gradients. The same trend is, however, observed in all the experiments. The physiologic opening of the eustachian tube reduces the ΔPCh–TC and the ΔPTC–PI, respectively. This causes a brief, transient fall of the PPI, quickly equilibrated through the patent cochlear aqueduct. The same phenomenon is observed when the PCh is lowered. Thus, the protective effect of the tubal opening — when the aqueduct is patent — effectively reduces the significant pressure gradient between the tympanic cavity and the ambient pressure. It also reduces the large pressure gradient toward the labyrinth.

In the fourth set of experiments (condition 4, Fig 5), the main route of pressure transfer between the perilymph and cerebrospinal fluid, the cochlear aqueduct, is surgically obstructed, as well as the eustachian tube. The initial fall of the PTC is limited by elastic factors, of which the capacity seems exhausted during the second lowering of the PCh.[21] The prolonged but limited fall of the PTC is most likely influenced by compression of the tympanic mucosa and redistribution of the tympanic gases.[22] These long-acting and slowly reversible factors are probably the main reason for the delayed return of the PTC to preexperimental levels after normalization of the PCh. The hypobaric conditions, as well as the ΔPCh–TC gradients after the first and second pressure reductions — at 300 and 600 seconds, respectively — are not different from the changes recorded with the cochlear aqueduct patent and the eustachian tube blocked ($p > .05$, unpaired *t*-test; Fig 7). The PPI changes are, however, different, as only limited pressure equilibration between the labyrinthine and the cerebrospinal fluids is possible when the cochlear aqueduct is blocked.[23,24] Thus, a limited fall of the PPI results in a large pressure gradient between the labyrinth and the cerebrospinal compartment that remains throughout the experiment. The PPI fall is primarily hampered by PTC changes, but proves, nonetheless, significantly larger than the PTC fall. The explanation is some, but much restricted, equilibration to the PCSF through the parallel channels: endolymphatic duct and sac and possibly perivascular and neural pathways[23-25] entering the cochlea from the internal auditory meatus. The ΔPTC–PI is significantly smaller after the first and second pressure reductions, at 300 and 600 seconds, respectively ($p < .05$, unpaired *t*-test), than during conditions with the aqueduct patent (Fig 7). When the PCh is normalized, reversal of the pressure gradients between the PPI and the PCSF is observed. The PPI level remains lowered during the observation period of 5 minutes due to the previous redistribution of labyrinthine fluids.

In the last set of experiments (condition 5, Fig 6), the eustachian tube is physiologically patent, but the cochlear aqueduct is surgically blocked — a condition rare in cats but gradually common in humans with increasing age.[19,20] The individual pressure equilibration reduces the pressure gradient between the PTC and the PCh. This causes a substantial fall of the PPI and, consequently, a reduction of the pressure gradient between the labyrinth and the cerebrospinal fluid compartment until the PCh is normalized. Neither the PPI nor the PTC reaches preexperimental levels during the observation period after normalization of the PCh.

The study supports our views previously presented,[1,2] that the closed cavity of the middle ear is the prerequisite for the emergence of various pressure gradients between the tympanic cavity, the labyrinth, and their surroundings during hypobaric pressure.

The protective effect of the eustachian tube opening involves, therefore, not only the middle ear membranes but also the labyrinth and its contents, as all new pressure gradients are reduced (Figs 4 and 6). This is irrespective of the patency of the cochlear aqueduct.

When the aqueduct is blocked, the pressure equilibration from the labyrinth to the cerebrospinal fluid is seriously limited. Under these conditions, the pressure gradient toward the tympanic cavity across the labyrinthine window membranes is most often limited, but the gradient toward the cerebrospinal fluid compartment is generally large (Figs 5 and 6). As the endolymphatic sac in the posterior fossa is directly exposed to the PCSF (Fig 1), the membranous labyrinth is continuously affected by the large ΔPPI–CSF during hypobaric exposure. However, in cats this route of pressure transfer is limited and delayed.[24]

The equilibration of labyrinthine pressure through the patent cochlear aqueduct quickly eliminates any pressure difference between the labyrinth and the cerebrospinal fluid compartment. This induces, however, a large pressure gradient toward the tympanic cavity (Figs 3 and 4), of potential hazard to the labyrinthine window membranes (Fig 1).

Studies have shown that no significant pressure difference between the perilymph and endolymph exists in experimental animals during physiologic conditions.[26,27] There are, however, some reports in the literature of cochlear lesions during hypobaric conditions, eg, aviation. The damage mainly involves the fragile Reissner's and saccule membranes, as well as the labyrinthine window membranes.[28,29] Our study revealed no mechanical damage of the tympanic membrane nor the labyrinthine window membranes after exposure to limited levels of hypobaric pressure. We show, however, that maximum strain is exerted on the labyrinthine window membranes when the aqueduct is patent and the eustachian tube function is poor. Maximum strain on the membranous labyrinth is, however, likely to occur when the aqueduct is blocked and tubal function poor because of the large pressure gradient between the labyrinth and the cerebrospinal fluid compartment.

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INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

The biannual meeting of the Interamerican Association of Pediatric Otorhinolaryngology will be held in Sao Paulo, Brazil, September 21-24, 1997. For information, contact Office of the Secretary, Tania Sih, MD, PhD, Rua Itapeva, 366, Suite 102, 01332-000, Sao Paulo SP, Brazil; telephone 55 11 283-4645 or 283-3396; fax 55 11 826-9652 or 542-6037.

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EXTRACELLULAR FLUID PATHWAY INSIDE THE FACIAL NERVE FASCICLES

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The extracellular fluid pathway in the facial nerve and the diffusion of a tracer from the facial nerve to other cranial nerves was examined in the rabbit. Sodium fluorescein solution was injected into either the facial nerve fascicles or the epineurial connective tissue as a tracer at the stylomastoid foramen and then localized by fluorescence microscopy. In the facial nerve, fluorescence was observed in the endoneurium and external nerve sheath (epineurium and perineurium) through the geniculate ganglion following injection into the nerve fascicles. The vestibular, trigeminal, and glossopharyngeal ganglia also showed fluorescence on the injection side in ganglion cells and intercellular connective tissues. The results suggested that the endoneurial connective tissue constitutes a diffusion pathway inside the facial nerve fascicles and that the extracellular fluid pathway from the facial nerve to these cranial ganglia may be related to the neural spread of inflammation or neoplastic metastasis.

KEY WORDS — cranial ganglion, diffusion pathway, endoneurial fluid, extracellular matrix, facial nerve.

INTRODUCTION

Numerous studies have shown that herpes simplex virus travels up the axons of sensory nerves to the ganglion or central nervous system following inoculation of the cornea or foot-pad with the virus. Although subsequent demyelination in the central nervous system has been shown after these experimental procedures, there is no destruction or demyelination in the peripheral nervous system.[1,2] On the other hand, Townsend and Collins[3] demonstrated that demyelination in the peripheral nervous system is produced after direct injection of the virus into the peripheral nerve.

Meanwhile, it is known that Ramsay Hunt syndrome is caused by reactivation of varicella-zoster virus in ganglion cells and the spread to cranial nerves and cutaneous tissues. This syndrome consists of facial nerve paralysis and vestibulocochlear symptoms associated with vesicular eruption of the auricle and external auditory canal, and other cranial nerves, such as the trigeminal, glossopharyngeal, and vagus nerves, are occasionally involved. The mechanism of the spread of inflammation to these cranial nerves has not been sufficiently clarified.[4] The present study, therefore, was designed to examine the diffusion pathway within the facial nerve and the spread of dye from the facial nerve to other cranial nerves following direct injection of sodium fluorescein into the facial nerve of the rabbit.

MATERIAL AND METHODS

Injection of Sodium Fluorescein. Twelve healthy adult rabbits weighing about 2 kg each were used. The animals were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg body weight). After exposure of the extratemporal portion of the right facial nerve trunk under an operating microscope, 50 μL of 0.5% sodium fluorescein solution was injected into the nerve at the stylomastoid foramen with a microliter syringe. The solution was injected into the nerve fascicles in eight animals and into the epineurial connective tissue in four animals. Forty-five minutes after injection, the animals were painlessly sacrificed with pentobarbital and decapitated. Cranial nerves — the optic, trigeminal, oculomotor, facial, acoustic, glossopharyngeal, and vagus nerves — were exposed under the operating microscope and then removed.

Microscopic Examination. Specimens were frozen at –70°C without fixation. Sections were cut at a thickness of 4 μm with a freezing microtome and immediately examined with a fluorescence microscope without mounting.

DIFFUSION OF SODIUM FLUORESCEIN FOLLOWING INJECTION INTO FACIAL NERVE FASCICLES

Cranial Nerves Examined	*No. of Nerves With Fluorescence/Total*
Geniculate ganglion	8/8
Vestibular ganglion	7/8
Gasserian ganglion	2/8
Glossopharyngeal ganglion	2/6
Oculomotor nerve	0/4
Optic nerve	0/4

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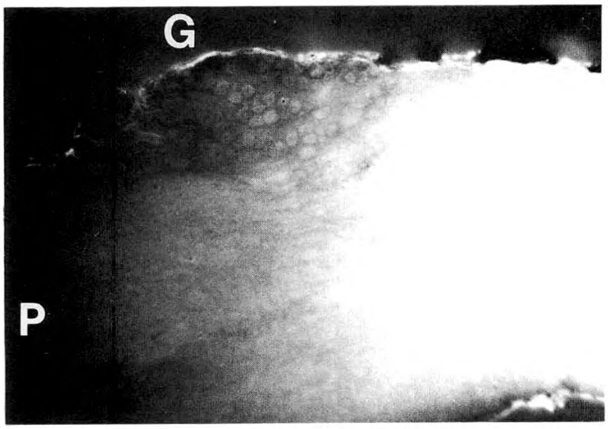

Fig 1. Fluorescence micrographs of facial nerve after injection of sodium fluorescein into nerve fascicles (original ×16). Fluorescence is visible in endoneurium and external nerve sheath through geniculate ganglion (G), but not in region (P) proximal to ganglion.

RESULTS

Animals receiving injection of fluorescein into the nerve fascicles showed diffusion in the endoneurium and external nerve sheath (epineurium and perineurium) along the nerve both distal and proximal to the injection point. The Table shows the fluorescence findings in cranial nerves following injection of fluorescein into the facial nerve fascicles. In the facial nerve, fluorescence was consistently observed through the geniculate ganglion, but it was not observed in the proximal nerve root (Fig 1). Seven of the eight vestibular nerves examined showed fluorescence in the ganglia (Fig 2). The trigeminal and glossopharyngeal ganglia also showed fluorescence in two of the six nerves examined (Fig 3). These ganglia showed fluorescence in ganglion cells and intercellular connective tissues only on the injection side. No fluorescence was observed in the optic or oculomotor nerves. Fluorescence in the spiral ganglion was undetectable because of the bony otic capsule.

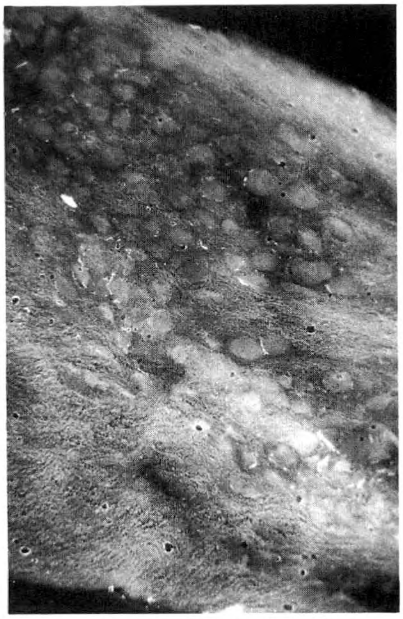

Fig 2. Fluorescence micrograph of vestibular ganglion after injection of sodium fluorescein into facial nerve fascicles (original ×40). Fluorescence is seen in ganglion cells and intercellular connective tissues.

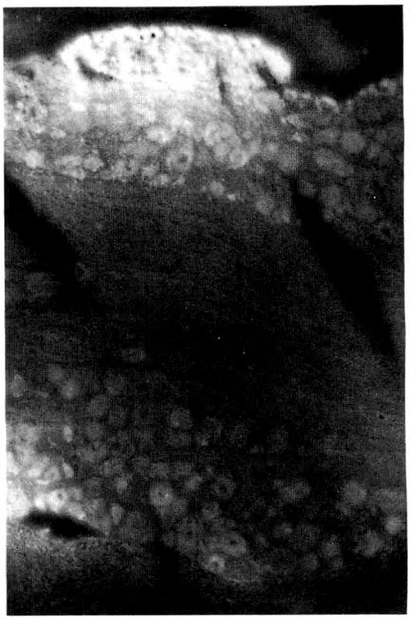

Fig 3. Fluorescence micrograph of glossopharyngeal ganglion (original ×25).

Animals receiving injection into the epineurial connective tissue, on the other hand, showed no fluorescence within the nerve fascicles despite an intense fluorescence in the external nerve sheath (Fig 4). No fluorescence was observed in these cranial ganglia.

DISCUSSION

Nerve fibers of the peripheral nerves are sur-

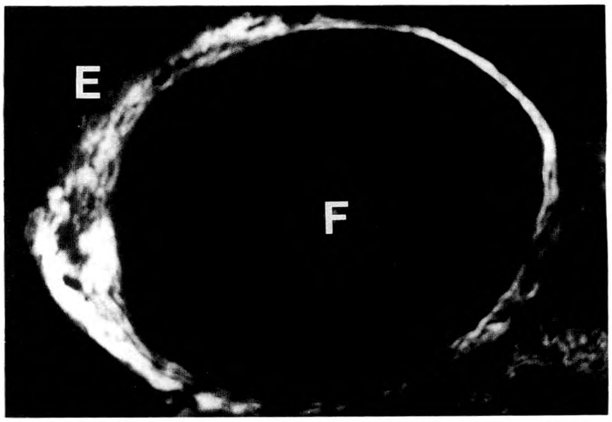

Fig 4. Fluorescence micrograph of facial nerve after injection of sodium fluorescein into epineurial connective tissue (original ×5). Fluorescence is present in external nerve sheath (E), but not within nerve fascicles (F).

rounded individually by endoneurial fluid and matrix, while the nerve roots run in the cerebrospinal fluid. This endoneurial fluid, an extracellular fluid inside peripheral nerve fascicles, forms the microenvironment of peripheral nerves and acts as a diffusion pathway.[5] The present study revealed the diffusion of fluorescein along the endoneurium and external nerve sheath following injection into the nerve fascicles, and showed no penetration across the perineurial diffusion barrier following injection into the epineurial connective tissue. These findings also suggest that the endoneurial connective tissue constitutes an extracellular fluid pathway within the facial nerve fascicles.

It is well known that varicella-zoster and herpes simplex viruses travel along the peripheral nerve and remain latent in sensory ganglia. These viruses are thought to travel up the axons of sensory nerves and take up residence in sensory ganglion cells. Although damage to the sensory root fibers is known to provoke reactivation of the virus, the mechanisms of this reactivation and the stimulation of viral movement to the periphery remain unknown.[6] It has been demonstrated that extracellular live virus is required for demyelination in the peripheral nervous system.[3] Therefore, the extracellular fluid pathway in this study seems to play an important role in the pathogenesis of viral neuritis in the peripheral nervous system.

In Ramsay Hunt syndrome, facial nerve paralysis and vestibulocochlear disorders are commonly observed, and other cranial nerves such as the trigeminal, glossopharyngeal, and vagus nerves are occasionally involved. Although anatomic connections in the cranial-cervical ganglionic chain have been considered,[7] the mechanism of the spread of inflammation to these cranial nerves has not been sufficiently clarified. The present study revealed a spread of dye not only into the geniculate ganglion but also into the vestibular, trigeminal, glossopharyngeal, and vagus nerves following direct injection of fluorescein into the facial nerve fascicles. Although the spread of fluorescein into the spiral ganglion was not recognized, the extracellular fluid pathway from the facial nerve to these cranial ganglia may be related to the neural spread of inflammation or neoplastic metastasis, eg, melanoma.

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END-TO-SIDE NEURORRHAPHY RESULTING IN LIMITED SENSORY AXONAL REGENERATION IN A RAT MODEL

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This study evaluated reinnervation of an end-to-side neurorrhaphy and the resultant functional recovery in a rat model. The cut distal posterior tibial nerve was repaired to the side of an intact peroneal nerve. In one group, the epineurium of the peroneal nerve was left intact; in another group, the epineurium was stripped; in the third experimental group, a perineurial slit was created. Evaluations included walking track analysis, nerve conduction studies, muscle mass measurements, retrograde nerve tracing, and histologic evaluation. Walking tracks indicated poor functional recovery. No significant difference in nerve conduction between the experimental and control groups was seen. Gastrocnemius muscle mass measurements revealed no functional recovery in the end-to-side groups. Retrograde nerve tracing revealed minimal staining of motor neurons. However, sensory neuronal staining of the dorsal root ganglia occurred in all groups. Histology revealed minimal myelinated axonal regeneration. These results suggest that predominantly sensory neural regeneration occurs in an end-to-side neurorrhaphy at an end point of 16 weeks.

KEY WORDS — end-to-side neurorrhaphy, horseradish peroxidase, Lewis rat, nerve conduction, peripheral nerve regeneration, walking track analysis.

INTRODUCTION

Traditional end-to-end neurorrhaphy allows recovery following nerve injury. Frequently, however, a reliable, expendable, or adequate source of sensory or motor nerve tissue is not available. Recent reports have suggested that end-to-side neurorrhaphy, in which the distal portion of an injured nerve is sutured to the side of the intact nerve, results in nerve regeneration.[1-8] Spontaneous regeneration from the intact nerve, neural contamination from the proximal stump of the divided nerve, and direct axonal injury to the intact nerve have been cited as potential explanations for this finding.

The purpose of this study was to investigate end-to-side neurorrhaphy with care to control potential contamination from the proximal nerve stump of the divided nerve and to control the preparation of the intact nerve.

MATERIALS AND METHODS

Animal Model and Design. Fifty adult male Lewis rats (Harlan Sprague-Dawley, Indianapolis, Ind) weighing 175 to 200 g were housed in flat-bottomed cages and fed standard rat chow and water ad libitum. The experimental design is shown in Fig 1. The 50 rats were randomized to one of five groups (n = 10 per group). Group 1 underwent a cut-and-repair of the posterior tibial nerve and served as the control group for functional recovery of the posterior tibial nerve. In group 2, an end-to-end neurorrhaphy of the peroneal nerve to the posterior tibial nerve was performed and served as the control for peroneal nerve regeneration. Groups 3 through 5 had an end-to-side neurorrhaphy of the distal posterior tibial nerve to the side of the peroneal nerve. The peroneal nerve was not prepared in group 3; a window of epineurium was stripped from the side of the peroneal nerve in group 4; and a longitudinal perineurial slit was created prior to repair in group 5 (Fig 1). Assessment of these

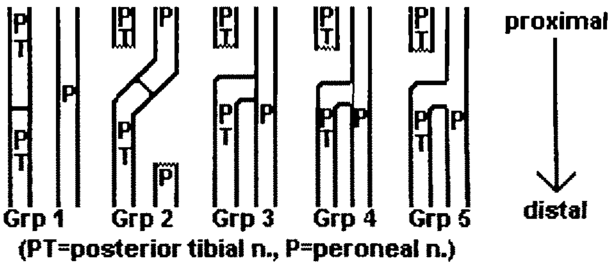

Fig 1. Experimental design.

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groups included serial walking track analysis, electrophysiologic studies, retrograde nerve tracing, muscle mass measurements, and histologic and morphometric analysis. The temporal end point for this study was 16 weeks.

Surgical Model and Technique. Anesthesia was induced with a subcutaneous injection of a mixture of ketamine hydrochloride (50 mg/kg), xylazine hydrochloride (10 mg/kg), and acepromazine maleate (1 mg/kg). The surgical procedures were carried out by means of standard microsurgical technique. The right sciatic nerve was approached via a standard dorsal gluteal-splitting incision, and the posterior tibial and peroneal nerve branches were then isolated. In group 1, a cut-and-repair of the posterior tibial nerve was performed with an epineurial technique and four 10-0 nylon sutures. In group 2, the posterior tibial and peroneal nerve branches were sharply transected and an end-to-end neurorrhaphy was performed between the proximal peroneal nerve and the distal posterior tibial nerve. In groups 3 through 5, the posterior tibial nerve was again sharply transected. The distal portion was then repaired to the side of the peroneal nerve, as described above, with three sutures. In groups 2 through 5, the proximal posterior tibial nerve stump was carefully buried in the gluteal muscles and sutured in place to prevent spontaneous regeneration from the proximal stump. The gluteal muscles were then reapproximated with a 4-0 absorbable suture, and the skin was closed with a running 4-0 nylon suture. The normal, unoperated, left sciatic nerve served as an intra-animal control. The peroneal nerve was left intact and the posterior tibial nerve was divided in order to allow for walking track evaluation, as division of the peroneal nerve would result in footdrop, which would make evaluation more difficult.[9]

Walking Track Analysis. Walking track analysis was used to evaluate rat hind-footprint patterns as previously described.[9,10] The footprint length (print length; PL) was then hand-measured on experimental (EPL) and control (NPL) sides. The PL factor (PLF) was then calculated as follows: (EPL – NPL)/NPL and was used for statistical comparison. All rats underwent serial walking track analysis every 2 weeks until sacrifice, beginning 4 weeks postoperatively.

Electrophysiologic Studies. At the time of sacrifice, nerve conduction studies were performed with a computer-assisted electromyographic machine (Advantage Medical Inc, London, Canada). The animals were anesthetized and the right and left sciatic nerves exposed. On the experimental (right) side, the sciatic nerve was exposed 2 cm proximal to the repair site and 0.5 cm distal to the site. A tracheotomy was then performed and the animal paralyzed with tubocurarine chloride (0.01 mg/kg). The animal was then kept on mechanical ventilation while temperature and heart rate were monitored. Bipolar electrodes (Nicolet Biomedical Instruments, Madison, Wis) were placed under the sciatic nerve proximally and under the posterior tibial nerve distal to the repair site. A ground wire was placed in muscle midway between the two electrodes. A total of three to seven averaged direct compound action potentials were obtained for each nerve. An identical setup, but on the contralateral (left) sciatic nerve, was performed to obtain intra-animal control data.

Histologic and Morphometric Studies. Following electrophysiologic studies, the nerve specimen was excised, including portions 1.5 cm proximal (peroneal nerve) and 1.5 cm distal (posterior tibial nerve) to the nerve repair site. The nerve was then fixed in situ in 2.5% glutaraldehyde. On the contralateral side, segments of normal posterior tibial and peroneal nerves were taken for control data. The nerves were then postfixed with osmium tetroxide and embedded in Araldite 502 (Polysciences Inc, Warrington, Pa). One-micrometer sections were stained with toluidine blue for light microscopy. Slides of the nerve were then evaluated to assess overall nerve architecture, quality and quantity of regenerated nerve fibers, fiber widths, percent of neural tissue, and density.

Quantitative histologic examination was then performed. At 1,000× magnification, six representative fields were evaluated per nerve by means of a digital image analysis system linked to morphometry software (Leco Instruments, St Joseph, Mich). Electron microscopy was also performed on the distal posterior tibial nerve from group 4.

Muscle Mass. The extensor digitorum longus (EDL) muscle, innervated by the peroneal nerve, and the gastrocnemius muscle, innervated by the posterior tibial nerve, were removed from the experimental and control sides in each group. A wet muscle mass was obtained, and a gastrocnemius and EDL ratio was calculated by dividing the experimental side by the normal side.

Horseradish Peroxidase and Fluoro-Gold Labeling. Four rats from each of groups 2 to 5 underwent horseradish peroxidase (HRP) and fluoro-gold (FG) retrograde nerve tracing. After the animals were adequately anesthetized, the sciatic nerve on the experimental (right) side was carefully exposed and the distal posterior tibial nerve dissected free. The nerve was then crushed and injected 5 mm distal to the repair site via micropipet with 3 to 5 μL of 10% type VI HRP (Sigma, St Louis, Mo) with care to avoid spillage. The proximal posterior tibial nerve

TABLE 1. PRINT-LENGTH FACTORS

	4 wk	*16 wk*
Group 1	0.423 ± 0.079	0.279 ± 0.097
Group 3	0.768 ± 0.112	0.836 ± 0.195
Group 4	0.542 ± 0.086	0.507 ± 0.096
Group 5	0.675 ± 0.179	0.676 ± 0.121

Data are mean ± SD.

was then dissected free from the muscle and injected in an identical manner with 3% FG (Fluorochrome Inc, Englewood, Colo).[11-13] The wounds were irrigated and closed. An identical procedure was then carried out on the left (contralateral) side with the peroneal nerve injected with 10% HRP and the posterior tibial nerve with 3% FG to serve as intra-animal controls.

After 48 hours, the animals were again anesthetized and were transcardially perfused with 100 mL of 0.9% saline, followed by 500 mL of 4% paraformaldehyde/0.25% glutaraldehyde in 0.1 mol/L sodium acetate buffer (pH 6.5), followed by 4% paraformaldehyde in 0.1 mol/L sodium borate buffer (pH 9.5), followed finally by 10% sucrose in 0.1 mol/L Sorenson's phosphate buffer.[14] The lumbar dorsal root ganglia (DRG) were carefully removed bilaterally at levels 4 and 5. The lumbar spinal cord was harvested as well. The lumbar spinal cord and DRG were sectioned longitudinally at 40 μm on a freezing microtome and collected in phosphate buffer. These sections were then reacted with hydrogen peroxide and tetramethyl benzidine to demonstrate HRP.[11] Sections were then serially mounted on glass slides, dried, and coverslipped with DPX mounting medium (Electron Microscopy Sciences, Ft Washington, Pa) without counterstain to minimize background fluorescence. These cells were then counted with a digital image analysis system linked to morphometry software (Leco Instruments). A ratio was created by dividing the experimental-side counts by the normal-side counts.

Statistical Analysis. Analysis of the differences of group means was calculated by a one-way analysis of variance followed by a post hoc Tukey's significant difference test for electrophysiologic conduction velocity, muscle mass ratios, labeled HRP nerve cell counts, and nerve fiber morphometry. The walking track analysis was compared by paired *t*-tests for dependent variables. All statistical tests were two-tailed and calculated with Statistica v4.5 (Statsoft Inc, Tulsa, Okla) software programs for personal computers.

RESULTS

Walking Track Analysis. Following division of the posterior tibial nerve, the rat's print length elongates, and as functional regeneration occurs, it shortens.[9,10] Division of the peroneal nerve results in a footdrop, in which the animals walk on the dorsum of their feet, making footprint measurements impossible. Thus, walking tracks from group 2 could not be analyzed due to unmeasurable tracks. Analysis of walking tracks was carried out at 4 weeks and 16 weeks in groups 1, 3, 4, and 5. The PLFs were calculated for each group as previously described and are presented in Table 1.

TABLE 2. MEAN CONDUCTION VELOCITIES

	Conduction Velocity (m/s)
Control	48.2 ± 3.09
Group 2	42.4 ± 6.54
Group 3	49.2 ± 5.14
Group 4	42.1 ± 3.81
Group 5	45.3 ± 5.67

Data are mean ± SD.

When statistical comparison was made between the 4-week and 16-week data, only control group 1 had statistically significant shortening of its PLF ($p < .05$). The experimental groups had no significant change in their PLFs.

Electrophysiologic Assessment. Mean conduction velocities were determined for groups 2, 3, 4, and 5 and are shown in Table 2. Three normal conduction velocities were taken from each group to serve as control data. There was no significant difference in conduction velocities between groups or the control data.

Muscle Mass Measurements. The EDL ratio was obtained for groups 1, 2, 3, 4, and 5. Group 2 served as a denervation control for the EDL ratio (Table 3). A statistical difference was seen only between group 2 and the remaining groups ($p < .01$). There was no significant difference between experimental groups 3, 4, and 5.

The gastrocnemius ratio was calculated for each group (Table 3). That for group 1, which had a cut-and-repair of the posterior tibial nerve, was significantly greater than that of all other groups ($p < .05$). Similarly, the gastrocnemius ratio for group 2 (end-to-end peroneal nerve to posterior tibial nerve) was

TABLE 3. MUSCLE MASS MEASUREMENTS

	EDL Ratio	*Gastrocnemius Ratio*
Group 1	1.030 ± 0.031	0.754 ± 0.040
Group 2	0.642 ± 0.180	0.568 ± 0.960
Group 3	0.947 ± 0.089	0.265 ± 0.115
Group 4	0.929 ± 0.094	0.234 ± 0.075
Group 5	0.914 ± 0.001	0.259 ± 0.096

Data are mean ± SD.
EDL — extensor digitorum longus.

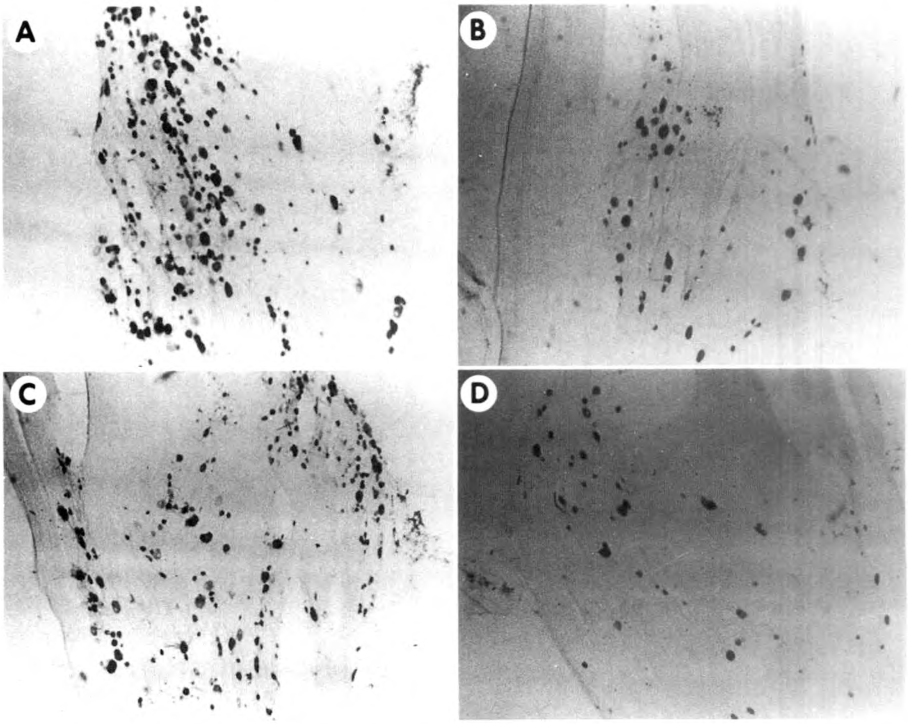

Fig 2. Examples of horseradish peroxidase–labeled dorsal root ganglia from lumbar level 4 (original ×4). A) Group 2 control side. B) Group 2 experimental side. C) Group 4 control side. D) Group 4 experimental side.

significantly greater than that for the experimental end-to-side groups, groups 3 to 5 ($p < .01$). However, there was no statistical difference between groups 3, 4, and 5.

Horseradish Peroxidase and Fluoro-Gold Labeling Results. Qualitative assessment of motor neurons within the lumbar spinal cord revealed positive labeling in control group 2 (end-to-end peroneal nerve to posterior tibial nerve), whereas assessment within experimental groups 3 to 5 revealed only minimal motor neuron labeling in groups 4 and 5, with no staining in group 3 on the experimental side. The absolute count averaged 7 motor neurons in group 4 and 16 motor neurons in group 5. However, when the DRG of groups 2 to 5 were reviewed, labeling occurred in all groups (Fig 2). The mean DRG neuron count ratios are presented in Table 4. There was no statistical difference between groups 3 to 5; however, all experimental groups had significantly lower ratios than control group 2 ($p < .01$).

Fluoro-gold labeling revealed normal distributions on the experimental and control sides in all groups. There was no double labeling of cells within the DRG or the lumbar spinal cord of any group.

TABLE 4. HORSERADISH PEROXIDASE LABELING

	Mean DRG Ratio
Group 2	0.905 ± 0.279
Group 3	0.140 ± 0.029
Group 4	0.207 ± 0.062
Group 5	0.176 ± 0.107

Data are mean ± SD.
DRG — dorsal root ganglion.

Histomorphometry. Qualitative assessment of nerve regeneration in the distal posterior tibial nerve of group 2 revealed many myelinated axons (Fig 3). In contrast, assessment of groups 3 to 5 revealed minimal myelinated axons dispersed among collagen fibrils with thickened perineurium (Fig 3). Quantitative analysis was performed, and total myelinated nerve fiber counts, fiber width analysis, percent neural tissue, and density were calculated (Table 5). There was no significant difference between groups 3, 4, and 5 in any of the histomorphometric parameters. However, all had significantly lower values than group 2 ($p < .01$). Electron microscopy was performed at 19,000× on the distal posterior tibial nerve from group 4 and again demonstrated few myelinated axons, but revealed a population of unmyelinated axons (Fig 4).

DISCUSSION

While end-to-side vascular repair is an established technique, the same concept used for nerve repair is highly unorthodox.[15] Ballance et al[16] were the first to report using an end-to-side neurorrhaphy in 1895 for the treatment of facial palsy secondary to ear disease. Others have described the use of an end-to-side repair for reinnervation in the treatment of brachial plexus injuries.[17,18] It was concluded, however, that a partial injury of the donor nerve was necessary for regeneration to occur, as has been recommended for the partial hypoglossal-to-facial nerve transfer for facial reani-

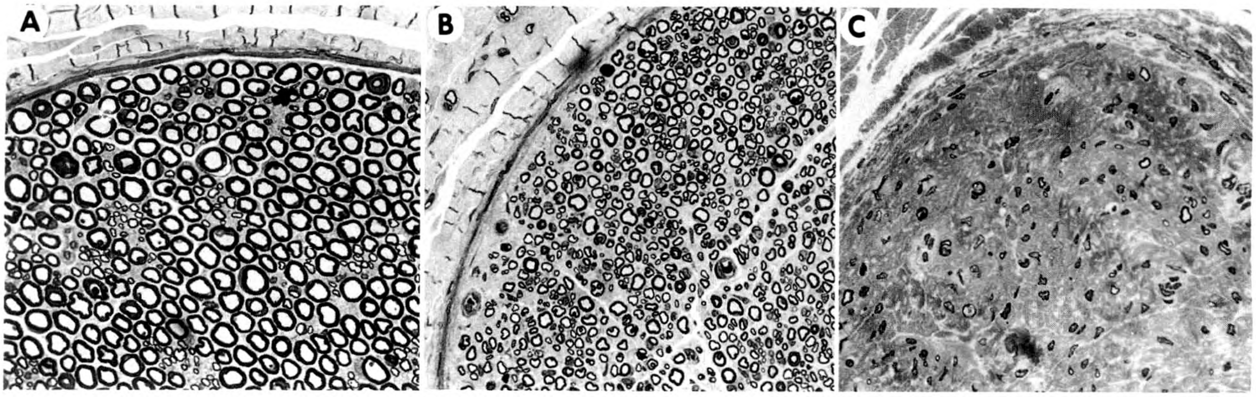

Fig 3. Original ×40. A) Normal posterior tibial nerve. B) Group 2 distal posterior tibial nerve. C) Group 4 distal posterior tibial nerve.

mation.[19]

Recently, Viterbo et al[1-4] and others[5-8] have reinvestigated this type of neurorrhaphy. Viterbo et al[1,3] studied end-to-side nerve repairs grafting the distal peroneal nerve to the side of the posterior tibial nerve with and without removal of the epineurium. Further work utilized a nerve graft with two end-to-side neurorrhaphies, with recovery demonstrated in all of these studies.[1,3,4] Viterbo[2] also carried this work to the clinical arena by using the technique for cross-face nerve transplantation for facial reanimation and for brachial plexus reconstruction. Results were promising in only one patient, and follow-up was early for evaluation of the five patients reported. Lundborg et al[5] explored this topic further by attempting to determine whether a degenerated nerve segment could induce nerve sprouting. Assessments included the "pinch" test, isometric contractility testing, and electromyography (EMG). It was concluded that both sensory and motor neurons regenerated. Sensory neurons regenerated better through a predegenerated nerve than across a fresh graft in the no-window group.

Casella et al[6] recently presented their work on end-to-side nerve repairs using the rat sciatic nerve model. An epineurial window was created prior to repair, and two groups were created for comparison, one with a direct end-to-side attachment and one with an end-to-side repair of an interposing nerve graft. Evaluations included walking track analysis, histology, and limited HRP retrograde nerve tracing. Neural regeneration was reported to be equally satisfactory in both groups.

Recently, Ross et al[7] presented their work with end-to-side nerve coaptation using New Zealand white rabbits. Comparison was made between a cut-and-repair of the musculocutaneous branch of the median nerve and experimental groups in which the cut musculocutaneous nerve was attached to the side of the median nerve in an end-to-side fashion. Half of the animals underwent immediate repair, and the other half had a delay of 4 weeks prior to coaptation. Neural regeneration with subsequent reinnervation of denervated muscle was found and was comparable to the nerve function of the control. No difference was seen between the two experimental groups.

The present study was undertaken to more clearly delineate the type of nerve preparation technique that would facilitate regeneration and to determine whether axonal damage to the donor nerve may have occurred and subsequently recovered by the time of sacrifice in the previous studies. In this study, the distal posterior tibial nerve was attached to the side of an intact peroneal nerve. Peroneal nerve function was preserved to avoid the footdrop that is associated with injury to the peroneal nerve.[9] This allows functional evaluation to be determined from PLFs.[9,10] In preliminary work, the epineurium was stripped from the peroneal nerve in 5 animals, and in a second group of 5 animals, a perineurial slit was created. These groups underwent walking track analysis preoperatively, and just prior to sacrifice at 2 weeks. For the group with an epineurial window, the peroneal functional index[10] (PFI) preoperatively and at 2 weeks was

TABLE 5. HISTOMORPHOMETRY

	Total Fiber Counts	*Fiber Width (μm)*	*Percent Neural Tissue*	*Density (Fibers/mm²)*
Group 2	2,855 ± 1,282	4.33 ± 1.25	34.76 ± 13.08	17,574 ± 7,425
Group 3	327 ± 264	2.75 ± 0.28	3.05 ± 1.83	3,356 ± 2,437
Group 4	408 ± 443	3.01 ± 0.52	4.30 ± 3.08	4,294 ± 3,950
Group 5	536 ± 452	2.65 ± 0.22	4.67 ± 2.75	4,959 ± 2,632

Data are mean ± SD.

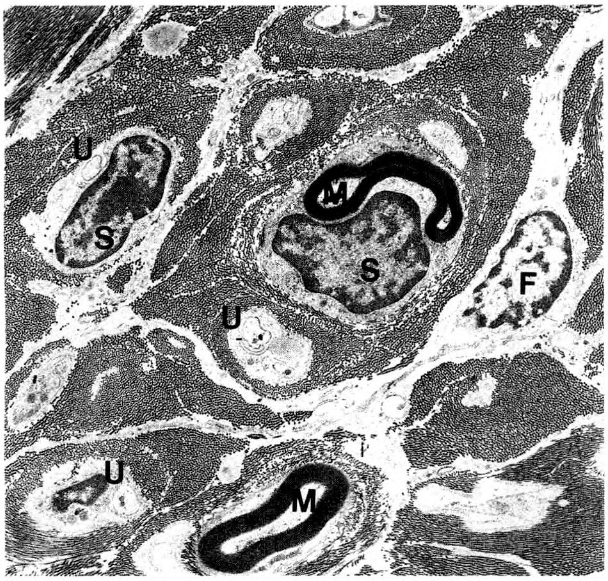

Fig 4. Electron microscopy of distal posterior tibial nerve of group 4 (original ×19,000). S — Schwann cell, M — myelinated axon, U — unmyelinated axon, F — fibroblast.

−4.41 ± 16.19 SD and −8.06 ± 14.36 SD, respectively. The PFI for the group with a perineurial slit preoperatively and at 2 weeks was −14.36 ± 9.42 SD and −14.94 ± 5.58 SD, respectively. Statistical comparison of the PFI revealed no significant differences, suggesting no significant functional injury to the nerve with these techniques. Qualitative histologic evaluation revealed minimal wallerian degeneration in the epineurial strip group (less than 5%), whereas in the perineurial slit group, there were approximately 10% to 15% of axons undergoing wallerian degeneration at 2 weeks.

In this study, neural regeneration was seen in all groups. It was consistent with predominantly sensory regeneration at this sacrifice point without any functional motor recovery. There is a direct correlation between wet muscle mass and isometric contractility of a reinnervated gastrocnemius muscle[20] (also unpublished observations, W. Kuzon et al). Thus, measurements of wet muscle mass correlate with measurements of muscle function. In this study, the results revealed significantly less muscle mass in the experimental end-to-side groups than in control group 2, but no difference among the experimental groups. Measurement and comparison of EDL muscle masses revealed no differences among groups and again showed no functional damage to the donor peroneal nerve. The fact that predominantly sensory regeneration was seen in this study is in contrast to the work of Lundborg et al[5] and Viterbo et al.[1,3] Lundborg et al performed muscle evaluation with isometric contractility testing and showed that the reinnervated tibialis anterior muscle was functioning at 60% of normal. In the work of Viterbo et al, EMG was performed, and it was stated that recovery was seen in 75% of animals and that the EMG signal could be terminated by posttest sectioning of the nerve. In these studies, however, there may have been distal contamination by regeneration of nerve fibers from the proximal stump, as suggested by Lundborg et al.

A concern with end-to-side neurorraphy has been whether regeneration occurs secondary to collateral sprouting at the site of the neurorrhaphy, at the level of the spinal cord, or from the proximal stump of the recipient nerve. For this reason, retrograde nerve tracing was undertaken with HRP and FG. The HRP labeled the distal posterior tibial nerve, and the FG labeled the proximal posterior tibial nerve stump. The HRP labeling revealed stained cells within the DRG and minimal labeling within the motor neuron strip of the spinal cord in groups 4 and 5, thus demonstrating predominantly sensory regeneration through the end-to-side repair. The proximal stump of the posterior tibial nerve was injected with FG. If collateral sprouting from the posterior tibial nerve neurons occurred at the spinal cord level, then double labeling would have been seen within the DRG. Because of the crush injury to the proximal stump of the posterior tibial nerve that occurred at the time of FG injection, all HRP injected into the distal posterior tibial nerve must have traversed the end-to-side repair, and thus, all HRP-labeled cell bodies must have been peroneal nerve in origin. However, no double labeling was seen, and HRP labeling occurred, indicating that collateral sprouting occurred from an intact peroneal nerve at the repair site. To further delineate the question of proximal stump contamination of the end-to-side repair, an additional 2 rats were studied with HRP labeling. The HRP was injected into the distal posterior tibial nerve, and the proximal peroneal nerve was cut sharply and buried into muscle. If contamination had occurred from the proximal stump of the posterior tibial nerve, then HRP would have traveled via this route and labeling would have occurred in the posterior tibial nerve distribution within the DRG and spinal cord. However, no labeling occurred on the experimental side; thus, it was confirmed that no contamination occurred from the proximal divided stump of the posterior tibial nerve.

It is of interest that predominantly sensory regeneration was found in all of the end-to-side groups at the temporal end point of 16 weeks, and that it was similar in all three groups. Although motor regeneration was minimal, it increased as manipulation of the donor nerve increased, ie, from group 3 to group 5. Motor recovery seen in other reports may have been related to contamination from the proximal divided nerve stump.

In conclusion, the induction of collateral sprouting from an intact nerve through an end-to-side neurorrhaphy would represent an important alternative for peripheral nerve reconstruction. However, the results of this study suggest that only poor sensory regeneration can be anticipated, so that clinical applicability is unlikely.

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APPLICATION OF OXYGEN FREE RADICAL SCAVENGERS TO DIMINISH THE OCCURRENCE OF MYRINGOSCLEROSIS

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The present study was designed to establish whether or not an increased production of oxygen-derived free radicals is involved in the causation of myringosclerosis. Sclerotic lesions in the tympanic membrane were experimentally elicited by keeping rats with perforated tympanic membranes in an atmosphere containing roughly 40% oxygen. The animals were treated daily with a solution containing either copper zinc–superoxide dismutase plus catalase, deferoxamine, or copper sulfate plus iron chloride, applied to the traumatized area. After 1 week the extension of myringosclerotic plaques was determined otomicroscopically. The pars tensa and pars flaccida were then dissected free and prepared for light microscopic studies. The results showed that treatment with copper zinc–superoxide dismutase plus catalase and deferoxamine inhibited or reduced the development of myringosclerosis, whereas the ears treated with copper sulfate plus iron chloride appeared unaffected. Consequently, the findings support the hypothesis that the formation of oxygen free radicals contributes significantly to the development of myringosclerosis.

KEY WORDS — catalase, hyperoxia, myringosclerosis, oxygen free radicals, superoxide dismutase, tympanic membrane.

INTRODUCTION

Myringosclerosis is a common sequela of ventilation tube treatment of otitis media with effusion.[1-8] The condition involves hyalinization and calcification of the collagen layer in certain areas of the tympanic membrane (TM).[9]

The middle ear cavity, in the human as well as in other animals, normally contains 5.5% to 12.1% oxygen (O_2).[10] Installation of a ventilation tube intrudes ambient air into the middle ear cavity, which results in an increase in the O_2 concentration.[11]

In a previous study, we showed that in rats kept in 40% O_2, perforation and intubation of the TM provoked the development of myringosclerosis compared with controls in ambient air.[12] We hypothesized that the pathogenesis of myringosclerosis might be due to an increased production of O_2-derived free radicals as a consequence of the hyperoxic condition in the middle ear cavity.

To test this possibility, we designed an experiment in which myringotomized rats, kept in 40% O_2, had their TMs treated with solutions containing substances affording protection from or enhancing the formation of O_2 free radicals. The scavengers used were copper zinc (CuZn)–superoxide dismutase (SOD), which dismutes the superoxide anion radical[13]; catalase, which dismutes hydrogen peroxide[13]; and deferoxamine, which reduces the formation of radicals by chelating iron ions.[14] To promote the formation of O_2 free radicals, iron and copper ions were added.

MATERIALS AND METHODS

Fifteen healthy male Sprague-Dawley rats, weighing 250 to 300 g, were anesthetized with methohexital (Brietal) via a tail vein. Under the otomicroscope, through a speculum, a perforation occupying the upper posterior quadrant of the TM was made in both ears with a myringotomy lancet. Twelve of the animals, kept in four cages with 3 animals in each, were placed in a Plexiglas chamber. For 1 week the animals were exposed to a flow of air and O_2, adjusted to a final O_2 concentration of 39% ± 2%. The gas concentrations in the chamber were analyzed twice daily.

Once a day, under Brietal anesthesia, the ears were treated according to the schedule in Table 1. We instilled 125 μL of the different solutions into the middle ear cavity, covering the perforated area. During the anesthesia, the status of the TM of the animals was ascertained under the otomicroscope. Prior to sacrifice, under Brietal anesthesia, the status of the TM was ascertained under the otomicroscope and photodocumented. The animals were then sacrificed painlessly with an overdose of pentobarbital (Mebumal) and decapitated. The middle ear cavity was opened and filled with a fixative solution containing

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TABLE 1. TREATMENT SCHEDULE

Group	*Treatment*	*No. of Treated Myringotomized Ears*
1	HEPES buffer (10 mmol/L sodium HEPES, 0.15 mol/L sodium chloride, pH 7.4)	4
2	HEPES buffer containing 30 µg/mL bovine copper zinc–superoxide dismutase (Boehringer Mannheim GMBH) plus 30 µg/mL bovine catalase (Boehringer Mamheim)	5*
3	HEPES buffer containing 0.1 mmol/L deferoxamine (Desferal, Ciba-Geigy)	5*
4	HEPES buffer containing 10 µmol/L copper sulfate plus 10 µmol/L iron chloride	5*
5	Nontreated ears kept in hyperoxia (ie, hyperoxic group)	5
6	Nontreated ears kept in ambient air (ie, normoxic group)	6

*Each group consisted of 3 rats. One ear of each group was left untreated and was included in hyperoxic group of ears.

TABLE 2. OTOMICROSCOPIC EVALUATION OF SCLEROTIC LESIONS OF PARS TENSA

	No. of Ears	0	+	++	+++
Superoxide dismutase plus catalase	5	2	2	1	
Deferoxamine	5	1	3	1	
Copper sulfate plus iron chloride	5		3	2	
Buffer	4		2	2	
Hyperoxia	5			4	1
Normoxia	6*		2	2	1

Horseshoe-shaped sclerotic pattern classified as follows: 0 — no visible sclerotic deposits, + — occasional lesions, ++ — moderate, +++ — grave.

*One tympanic membrane was covered with crust.

3% glutaraldehyde in a 0.075 mol/L sodium cacodylate buffer (pH 7.4) with 4% polyvinylpyrrolidone and 0.02 mol/L calcium chloride added. After immersion in the fixative for at least 24 hours at 4°C, the pars tensa and the pars flaccida of the TMs were removed. Immediately after dissection, the tissues were rinsed in cacodylate buffer and postfixed overnight in 1% osmium tetroxide. After rinsing, the specimens were dehydrated in a graded series of ethanol and embedded in Epon. The middle ear tissues were cut into 1-µm sections and stained with toluidine blue for studies under the light microscope. Thin sections were obtained for electron microscopic studies.

RESULTS

Otomicroscopically, the myringotomized nontreated ears kept in the hyperoxic atmosphere showed the most extensive occurrence of myringosclerotic plaques (Table 2). The term sclerotic plaque refers to the chalky areas that form a horseshoe pattern along the handle of the malleus and near the annulus, in the noninjured side of the pars tensa. The two groups of ears treated with buffer alone and copper sulfate ($CuSO_4$) plus iron chloride ($FeCl_2$), respectively, showed a moderate occurrence of sclerotic deposits. No significant difference was observed concerning the extension of the plaques when comparing these two groups. However, in the groups of ears treated with CuZn-SOD plus catalase and deferoxamine, two and one, respectively, of the TMs were devoid of sclerotic deposits. The other TMs from these two groups had only a slight appearance of myringosclerotic plaques, which were less pronounced than those in the ears treated with buffer alone and $CuSO_4$ plus $FeCl_2$.

Under the light microscope, the pars tensa specimens from the nontreated hyperoxic ears showed extensive sclerotic lesions, sometimes with a confluent appearance (Fig 1). The ears treated with $CuSO_4$ plus $FeCl_2$ or buffer alone showed a moderate occurrence of sclerotic plaques, comparable in frequency and extension to those seen in the specimens from the nontreated normoxic group of ears. In the specimens from the ears treated with CuZn-SOD plus catalase and deferoxamine, only a few isolated sclerotic deposits were seen in the collagen layer. On comparing the specimens from these two groups, more myringosclerotic lesions were noted in the deferoxamine group.

Three of the pars flaccida specimens from the nontreated hyperoxic group showed small, rounded sclerotic lesions localized along the inside of the squamous inner epithelium and occasionally in the stroma (Table 3 and Fig 2). More confluent sclerotic plaques were seen close to the inner epithelium. The lesions were most probably localized extracellularly. In the normoxic group, both confluent sclerotic plaques and rounded lesions were found in 3 of the specimens. In the buffer-treated group, a few confluent sclerotic plaques were found along the inner epithelium in 1 of the specimens. Sclerotic lesions were not visible in any of the specimens from the ears treated with $CuSO_4$ plus $FeCl_2$, CuZn-SOD plus catalase, or deferoxamine.

DISCUSSION

This study showed that application of solutions containing CuZn-SOD plus catalase and deferoxamine to traumatized TMs reduces the occurrence of myringosclerosis in rats kept in a hyperoxic environ-

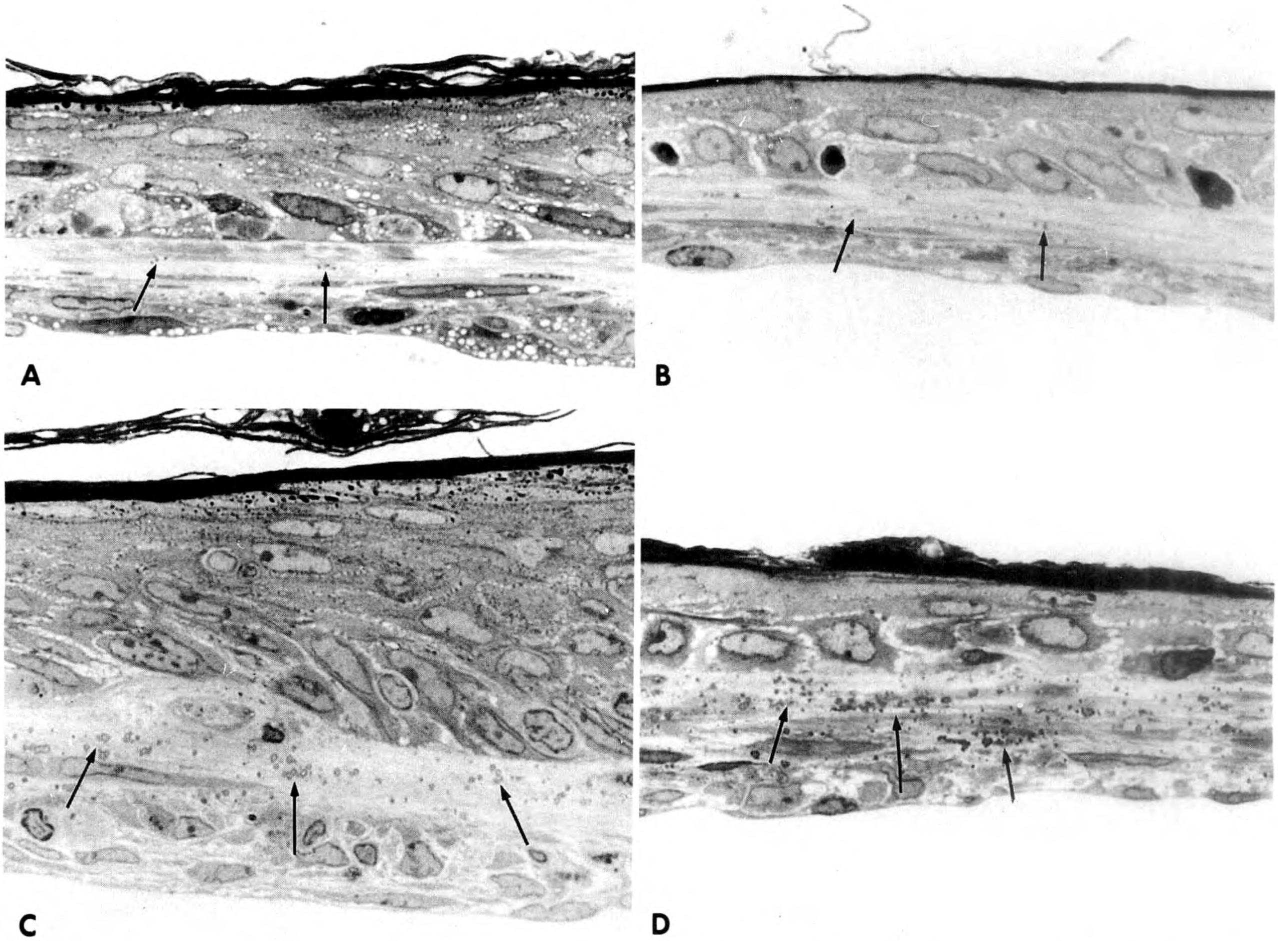

Fig 1. Area of pars tensa near malleus on nonperforated side of pars tensa (toluidine blue, original ×1,000). A) Pars tensa from copper zinc–superoxide dismutase plus catalase–treated ear. Note sparse sclerotic lesions in collagen layer (arrows). B) Pars tensa from deferoxamine-treated ear. Sclerotic lesions marked with arrows. C) Pars tensa from copper sulfate plus iron chloride–treated ear. Sclerotic lesions marked with arrows. D) Pars tensa from ear of untreated, hyperoxic group. Note abundant sclerotic deposits in collagen layer (arrows).

ment.

In previous reports, the histology findings of sclerotic lesions in the TM and the middle ear mucosa of humans, rats, and guinea pigs have been compared at both light and ultrastructural levels. The results show no difference regarding the structure and localization of the sclerotic plaques between various species.[9,15-21] The present myringosclerosis model is therefore regarded as comparable to the sclerotic lesions that develop after ventilation tube insertion in humans. Several hypotheses as to the cause of myringosclerosis have been formulated. Tos et al[7] proposed a mechanical theory. They claimed that the natural movement of a TM with inflammatory fibrous hyperplasia is impeded by the installation of a ventilation tube. The reduced mobility of the TM promotes hyalinization and calcification in the collagen layer. Other authors reported that excessive bleeding during aspiration of the middle ear fluid or during insertion of the tube is correlated to an increased development of myringosclerosis.[22-24] Yet another hypothesis suggested that myringosclerosis develops as a result of immunologic hypersensitivity in previously sensitized tissue.[20] It has also been shown that one can reduce the frequency and extension of the sclerotic deposits by treating rats with perforated TMs with the chelating agent citrate (0.05 mol/L citrate buffer, pH 6.0).[25]

TABLE 3. LIGHT MICROSCOPIC OCCURRENCE OF MYRINGOSCLEROSIS IN PARS FLACCIDA

	No. of Specimens	*No. With Myringosclerotic Lesions*
Superoxide dismutase plus catalase	5	0
Deferoxamine	5	0
Copper sulfate plus iron chloride	5	0
Buffer	4	1
Hyperoxia	5	3
Normoxia	5	3

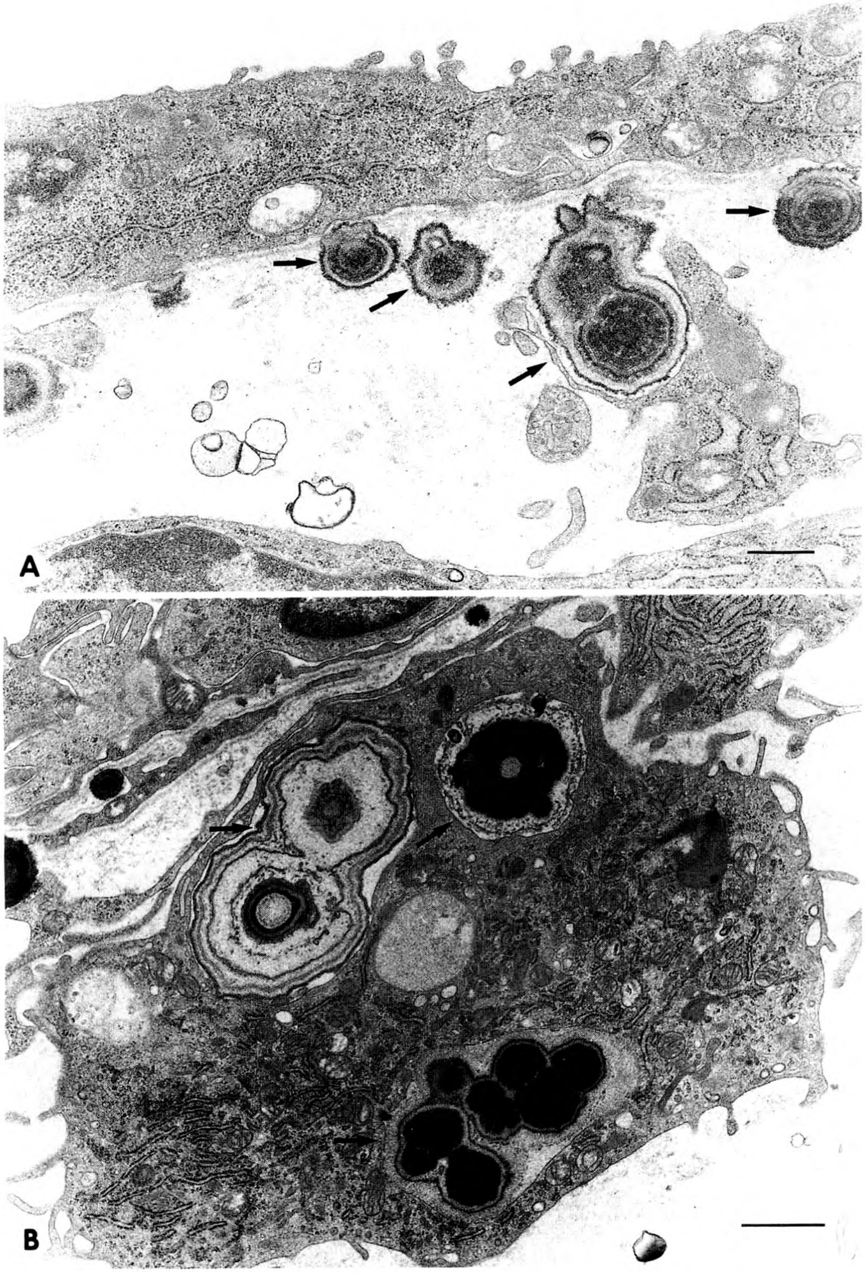

Fig 2. Electron micrographs of pars flaccida (toluidine blue). A) From ear of untreated hyperoxic group. Sclerotic lesions marked with arrows. Bar — 500 nm. B) Three sclerotic deposits with different appearance (arrows). Bar — 1 µm.

Recently, we established an animal model for experimental development of myringosclerosis.[12] By increasing the O2 concentration in the ambient atmosphere and subsequently in the middle ear cavity, an acceleration in the production of O2-derived free radicals can be elicited.[26] Their presence probably initiates the pathologic processes that are seen as myringosclerotic plaques in the TM.

Most of the O2 consumed in the body is reduced directly to water by the cytochrome oxidase complex of the mitochondria. However, in many systems in the body, O2 can also be reduced stepwise to form free radicals and other reactive species. Such reactions have been estimated to account for a minor proportion of O2 consumption.[13] To reduce the toxicity of these reactive species and O2 free radicals, the body has an elaborate system of enzymatic and nonenzymatic antioxidants. Nevertheless, tissue damage can occur when an excess of radicals is formed, eg, when cells are exposed to hyperoxia, during reperfusion after ischemia, and in inflammation when phagocytic cells are activated to produce large amounts of superoxide radicals.[13,27]

The protective effect of CuZn-SOD plus catalase in the present study suggests that superoxide radicals ($O_2^{\cdot -}$) and/or hydrogen peroxide (H_2O_2) are involved in the pathogenesis of myringosclerosis. The somewhat less distinct but still significant protection afforded by the iron chelator deferoxamine further suggests the involvement of iron ions in the process. Together, these results suggest that the hydroxyl radical (OH$^{\cdot}$) or some strongly oxidizing iron-oxygen species is the final toxic agent according to the following reactions.

$$\mathrm{O2}^{\cdot-} + \mathrm{Fe}^{3+} \rightarrow \mathrm{Fe}^{2+} + \mathrm{O2}$$

$$\mathrm{H2O2} + \mathrm{Fe}^{2+} \rightarrow \mathrm{Fe}^{3+} + \mathrm{OH}^{\cdot} + \mathrm{OH}^{-}$$

Copper ions can catalyze a similar set of reactions.[28] However, addition of copper and iron ions to the buffer did not potentiate the myringosclerosis, possibly due to the relatively low concentrations of the metal ions used, due to lack of penetration to the main site of action, or because sufficient amounts of the ions were already present. Another possibility, compatible with all results, is that peroxynitrite is the main toxic agent in the model. Peroxynitrite is formed by the reaction between superoxide and nitric oxide.[29]

$$\mathrm{O2}^{\cdot-} + \mathrm{NO}^{\cdot} \rightarrow \mathrm{ONOO}^{-}$$

Deferoxamine is an efficient direct scavenger of peroxynitrite,[29] and the CuZn-SOD would reduce peroxynitrite formation by scavenging the superoxide radical. Synthesis of nitric oxide can be induced in phagocytes and a variety of other cell types, eg, by inflammatory cytokines.[30]

The primary sources of O2 free radicals in the present experimental model could involve an enhanced radical formation in mitochondria and the endoplasmic reticulum[26] caused by hyperoxia, by oxygen radicals formed from activated phagocytes,[27] or as by-products of prostaglandin and leukotriene biosynthesis.[31]

The present study shows that the formation of myringosclerosis following experimental myringotomy can be reduced by treatment with a buffer of appropriate pH containing various antioxidants. This finding suggests that administration of antioxidants in combination with the insertion of ventilation tubes would also decrease the incidence of myringosclerosis in patients. However, the present study could not determine which antioxidants should be used, or at what concentrations. Such judgments must await further experiments.

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INTERNATIONAL PEDIATRIC OTOLARYNGOLOGY ENDOSCOPY DAYS

International Pediatric Otolaryngology Endoscopy Days of Lyon: The Pediatric Larynx will be held March 2-3, 1998, in Lyon, France. For more information, contact the Secretariat, Department ORL, Hopital E. Herriot, 69003, Lyon, France; fax 33-4-72-11-05-34.

IMAGING CASE STUDY OF THE MONTH

MELANOMA OF THE PETROUS APEX OF THE TEMPORAL BONE

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INTRODUCTION

Primary melanomas of the temporal bone are rare. Two cases of malignant melanoma of the middle ear have been reported recently.[1,2] Both of these cases were thought to arise from the mucous membrane lining the middle ear. Approximately 30% of all melanomas are found in the head and neck. The majority are cutaneous melanomas; however, mucosal melanomas are still a well-known entity. The temporal bone is a well-known site of metastatic involvement for many tumors, including melanoma.[3,4] A search of the English-language literature reveals no cases of primary involvement of the petrous apex by melanoma.

This report presents the first case of a primary melanoma of the petrous apex of the temporal bone. This case is unique in that the tumor appears to have originated in the petrous apex of the temporal bone with subsequent extension into the middle ear. The patient's history and relevant imaging and pathologic findings, as well as a discussion of the management of this unusual clinical scenario, are presented.

CASE REPORT

A 45-year-old man presented with a complaint of aural fullness, headache, and diplopia. Physical examination revealed an apparent mass in the middle ear and a sixth nerve palsy. Magnetic resonance imaging (MRI) showed an extensive mass involving the petrous apex (Fig 1). Computed tomography (CT) of the temporal bone showed a 2 × 3-cm mass of the petrous apex with extensive destruction of the tempo-

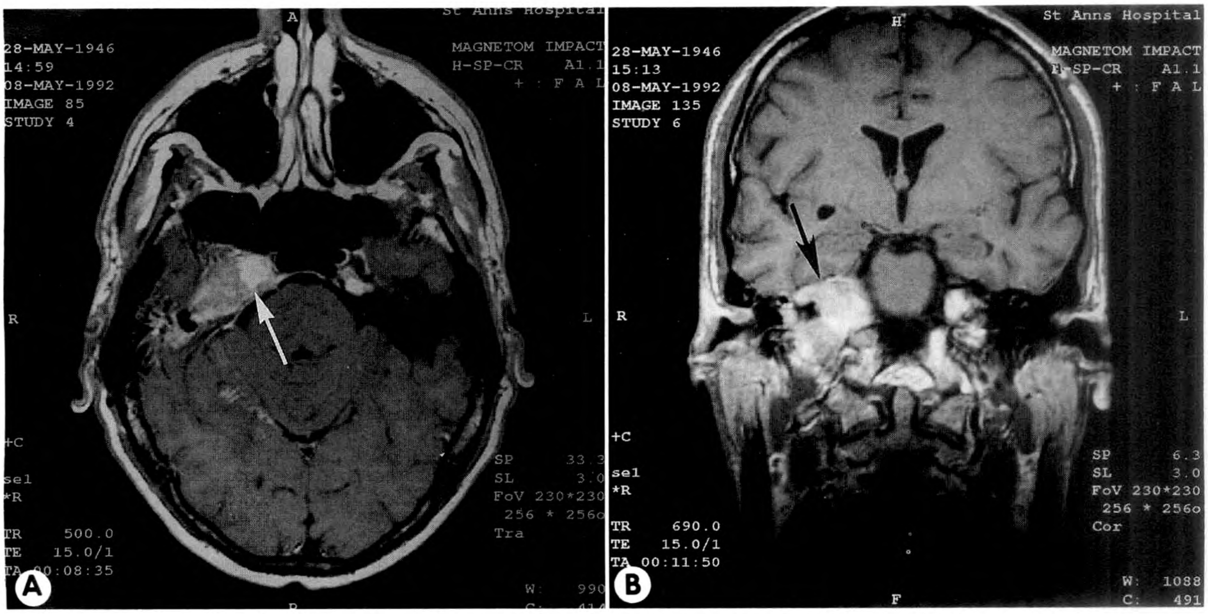

Fig 1. T1-weighted, gadolinium-enhanced magnetic resonance images of temporal bone, showing extensive lesion of petrous apex (arrows). Note irregular borders and inhomogeneous nature of lesion. A) Axial. B) Sagittal.

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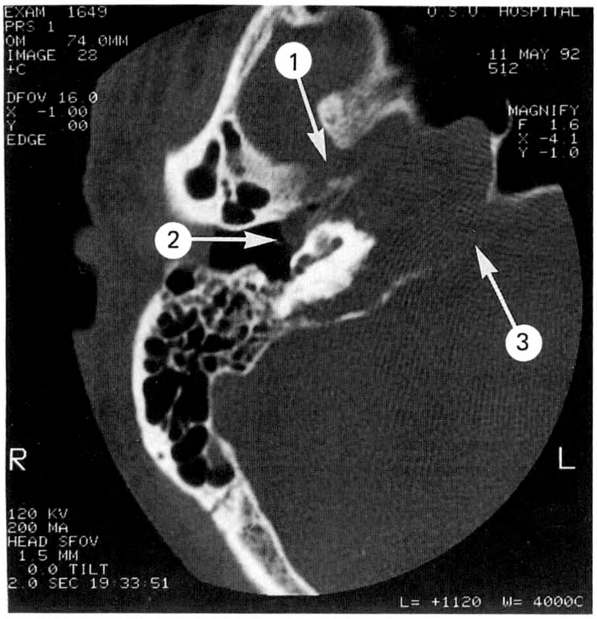

Fig 2. Axial computed tomogram of temporal bone showing lytic lesion of petrous apex along posterior carotid artery (1) with middle ear extension (2) and expansion of posterior fossa cortex with erosion medially (3).

ral bone (Fig 2). Arteriography showed this tumor to be a relatively avascular lesion.

The patient underwent an exploratory tympanotomy with biopsy of the middle ear portion of the mass. Pathologic analysis yielded a diagnosis of malignant melanoma (Fig 3).

To exclude metastatic melanoma, a thorough evaluation was carried out. The patient underwent a dermatologic examination revealing only a single pigmented lesion of the face. On excisional biopsy this was benign. Ophthalmologic examination revealed no evidence of retinal disease. We performed CT scans of the thorax and abdomen, as well as a bone scan, to rule out metastatic disease. No other lesions were identified. After oncologic consultation and extensive discussion with the patient and his family, the patient elected to undergo surgical resection of the tumor of the temporal bone including the petrous apex. The patient underwent preoperative balloon occlusion of the carotid artery, which he tolerated well.

The patient underwent an infratemporal fossa tumor resection. Tumor extended to the middle and posterior fossa dura. This dura was resected along with cranial nerves III through XII. Medially, the tumor extended into the cavernous sinus, involving the fourth and sixth cranial nerves, as well as the third division of the fifth nerve. Tumor also involved the carotid artery, necessitating carotid resection. The entire otic capsule, which was surrounded by tumor, was removed in toto. A fat graft and pericranial flap were used to reconstruct the defect.

Postoperatively, the patient experienced hoarseness and aspiration as a result of his 10th nerve deficit. A medialization thyroplasty was performed. On lumbar puncture, malignant cells were seen in the cerebrospinal fluid.

The patient recovered and was discharged 3 weeks postoperatively. Four months later he reported increasing headache. Repeat MRI revealed metastatic intracranial disease. The patient received a course of intrathecal thiotepa, systemic cisplatin, and palliative radiotherapy. He died at home 6 months after the initial diagnosis.

DISCUSSION

Melanoma, a malignant tumor of melanocytes, is derived from cells of neural crest origin. These cells

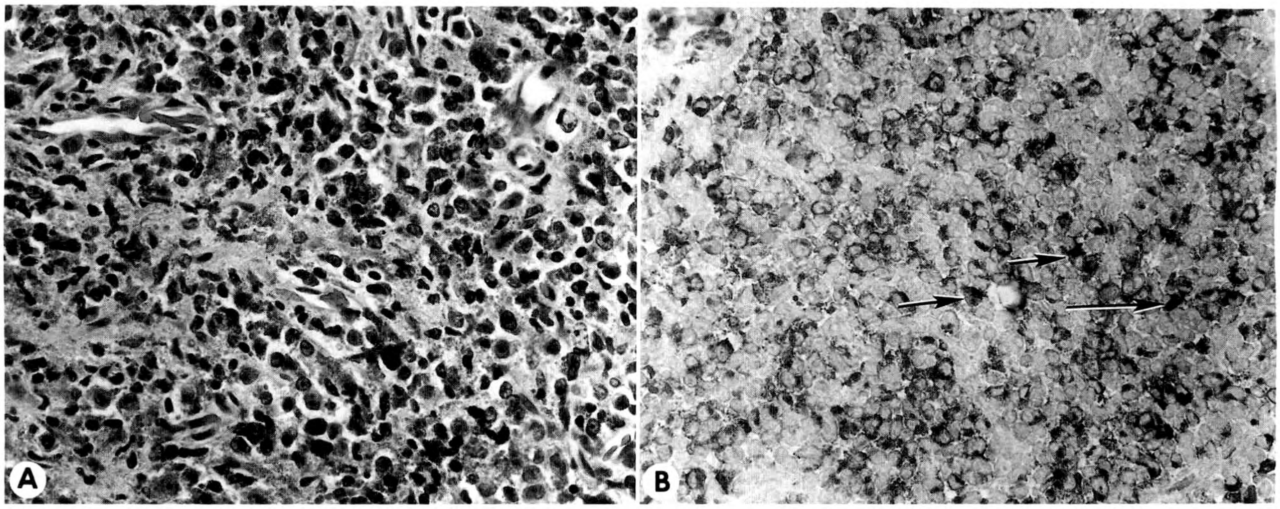

Fig 3. Photomicrographs of lesion. A) H & E, original ×400. B) Immunoperoxidase stain with typical staining (arrows) of lesion (HMB-45 stain, original ×300).

migrate during embryological growth, so that the greatest number are found at the dermal-epidermal junction of the skin. These cells are also found in other head and neck sites with mucosal-lined surfaces, such as the nasal cavity, paranasal sinuses, oral cavity, and pharynx.

Melanomas constitute 1.2% of all malignancies and are responsible for 0.74% of all deaths from cancer.[5] Over 90% of these occur on the skin, where epidemiologic studies have shown a rising incidence over the past 30 years.[6]

Carcinomas from various sites throughout the body are known to metastasize to the temporal bone — most frequently from the breast, kidney, and lung.[7] Likewise, melanoma is known to metastasize to the temporal bone, as well as numerous other sites in the body.[1] In contrast, primary melanomas of the temporal bone are exceedingly rare. To date, there have been two reported cases of primary melanoma of the temporal bone.[3,4] Both of these cases involved the middle ear as the primary site. This case demonstrated some minor middle ear extension; however, the epicenter of the lesion involved the cells of the petrous apex, with extensive bony destruction and invasion of major vascular and nervous structures.

The differential diagnosis of a petrous apex lesion is complex.[8,9] Neoplastic lesions usually reveal hypointense signals on T1-weighted MRI scans. Neoplastic lesions are usually hyperintense on T2 and T1 with gadolinium contrast, as demonstrated in this case.

Surgical removal is the mainstay of treatment for malignant melanoma, but melanomas originating from tissues other than skin have a much worse prognosis.[5] This case demonstrates how very extensive lesions of the temporal bone produce symptoms only late in the disease course. Before deciding on a treatment plan, it is important to discuss with the patient the poor prognosis that exists even with extensive resections and adjunctive irradiation or chemotherapy.

In summary, the first known case of primary melanoma of the petrous apex of the temporal bone is presented.

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IMAGING CASE STUDY OF THE MONTH

MONOLATERAL APLASIA OF THE PAROTID GLAND

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INTRODUCTION

Congenital absence of the major salivary glands, especially of the parotid gland, is a rare disorder whose etiopathogenesis is poorly understood. Aplasia of the parotid glands may be unilateral or bilateral and may occur alone or in association with the absence of other salivary glands or with other developmental anomalies of the first branchial arch such as hypoplasia or aplasia of the lacrimal glands, hemifacial microsomia, mandibulofacial dysostoses, and multiple congenital anomalies.[1-14]

CASE REPORT

A 38-year-old man presented in September 1992 with a 10-month history of a painless swelling in the right parotid region, arising without other significant symptoms or apparent causes. The swelling was gradually increasing, without signs or symptoms of homolateral facial weakness, and did not vary in size while he was eating. On examination there was a soft, diffuse, nontender swelling of the right parotid region without any nodules, with normal salivary flow. No primary tumors of the head and neck or cervical lymph node involvement were detectable. Ultrasound examination of the right parotid showed a homogeneous enlargement of the gland, with no evidence of stones, nodules, cysts, or tumors. T1-weighted magnetic resonance imaging (MRI) in axial (see Figure, A) and coronal planes (see Figure, B) showed unilateral enlargement of the right parotid gland without areas of altered signal intensity, and a total absence of the left parotid gland. Subsequent salivary gland scintigraphy with technetium-99m pertechnetate showed a total absence of uptake on the left parotid side, with normal uptake in the contralateral parotid gland and the other salivary and lacrimal glands (see Figure, C). The patient had no symptoms associated with the absence of the left parotid gland. On clinical reexamination the orifice of the left parotid duct was absent, the oral mucosa was dry, and the other major salivary glands were functional. The left hemifacial contour was normal, and there were no depressions in the left preauricular region. All other members of the patient's family examined had normal function of the parotid glands. He required no treatment. Follow-up at 37 months showed no clinical developments. He had no symptoms other than a persisting functional hypertrophy of the right parotid gland.

DISCUSSION

The true incidence of unilateral aplasia of the parotid gland is difficult to ascertain because it is often asymptomatic. There have been sporadic reports in the literature and only one case regarding a simple hypertrophy of the parotid gland with contralateral parotid aplasia (see Table[1-7,10,12-23]). The number of cases reported increases if we include also those with familial occurrence and those associated with other developmental anomalies of the head and neck.[3,4,6,8,9,11-13,15-28] In these cases the familial distribution indicates a simple autosomal dominant gene with a high degree of penetrance.

Congenital unilateral absence of the parotid gland usually presents as a unilateral painless swelling of the parotid region that increases progressively due to the compensatory hypertrophy of the contralateral parotid gland, which often alerts physicians.

Imaging is fundamental for diagnosis. Ultrasound imaging should be carried out first, on both sides, in order to rule out unexpected contralateral aplasia, particularly when the ultrasound appearance of the salivary gland is that of a homogeneous parenchyma, with no stones, nodules, cysts, tumors, or regional lymphadenopathy.[14] Coronal and axial computed

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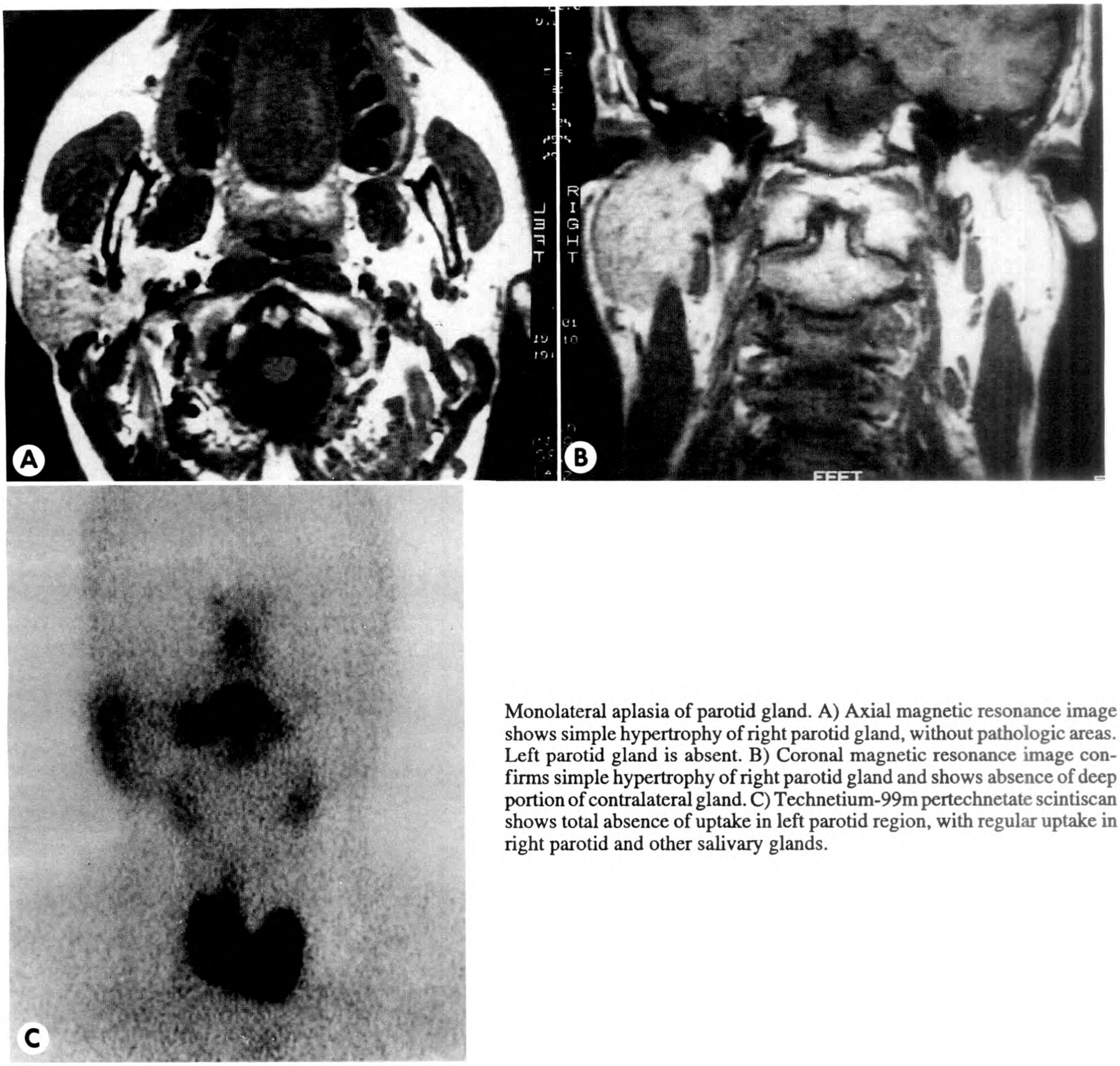

Monolateral aplasia of parotid gland. A) Axial magnetic resonance image shows simple hypertrophy of right parotid gland, without pathologic areas. Left parotid gland is absent. B) Coronal magnetic resonance image confirms simple hypertrophy of right parotid gland and shows absence of deep portion of contralateral gland. C) Technetium-99m pertechnetate scintiscan shows total absence of uptake in left parotid region, with regular uptake in right parotid and other salivary glands.

tomographic and MRI scans have a high sensitivity and specificity with regard to soft tissues, and permit a comparison between the two sides.[29-33] The coronal planes enable us to focus on the deep portion of the parotid gland with precision, and confirm the diagnosis of true aplasia. Salivary gland scintigraphy with technetium-99m pertechnetate is another simple, rapid, relatively safe, and noninvasive procedure, and is an indicator of salivary function, because pertechnetate is fixed and excreted by the glandular parenchyma.[21,22] Pertechnetate uptake by the salivary gland proves to be totally absent on the side of the aplasia, while a normal pattern is present in the contralateral, hypertrophic gland.[34,35] The presence of residual tissue of the agenetic salivary gland can be easily excluded.

Clinically, the absence of the orifice of the salivary duct can be taken as evidence of a developmental defect rather than atrophy subsequent to disease. When a unilateral swelling of the parotid gland is present, functional hypertrophy should also be considered, due to aplasia of the contralateral parotid gland. This finding may be totally misunderstood by the clinician, particularly if ultrasound imaging is carried out only on the enlarged parotid gland. A comparative ultrasound study of both parotid regions is to be recommended so as to rule out contralateral aplasia. Computed tomographic and MRI scans, together with technetium-99m pertechnetate scintigraphy, will confirm the diagnosis of unilateral aplasia, excluding the presence of residual and/or ectopic glandular tissue on the affected side.

REVIEW OF LITERATURE

Authors	*Year*	*Monolateral Parotid Gland Aplasia*	*Bilateral Parotid Gland Aplasia*	*Aplasia of Major Salivary Glands*	*Aplasia of Major Salivary Glands Associated With Aplasia of Lacrimal Glands*	*Aplasia of Major Salivary Glands Associated With Multiple Craniofacial Anomalies*
Gruber[1]	1885			1		
Bruno[2]	1894			1		
Ramsey[3]	1924			1		
Raison[5]	1925				1	
Blackmar[15]	1925				1	
Singer[4]	1925		1			
Nayak and Ananthanurayana[16]	1927		1			
Austin and Steggerda[6]	1936					1
Sharp[17]	1937		1			
Mutch[7]	1944				1	
Faber[18]	1942					1
Kabalov[19]	1950		1			
Rule[10]	1969		1			
Smith and Smith[20]	1977			2		
Cagamise and Townes[12]	1980				3	
Wiesenfeld et al[21]	1983		1			
Sucupira et al[22]	1983				1	
Wiesenfeld et al[13]	1985		1			
McDonald et al[23]	1986				1	
Kelly et al[14]	1990	1				

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CLINICOPATHOLOGICAL CONSULTATION

HEAD AND NECK LANGERHANS CELL HISTIOCYTOSIS

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Among the potential sites of involvement by Langerhans cell histiocytosis (LCH), the head and neck region is the most commonly cited. Though principally a pediatric disease, LCH can affect any age group. It can be unifocal (skeletal) or multifocal (skeletal and/or visceral); it appears as though the presence of visceral lesions is more common in the youngest patients, and may be associated in some with a rapidly progressive course resulting in death. Head and neck manifestations may mimic such varied entities as eczema, otitis media, osteomyelitis, and cholesteatoma. Current approaches to therapy are less aggressive than they were in the past, and are particularly intended to monitor for and treat any complicating secondary infections (which may develop in the youngest patients with multifocal disease including visceral involvement). The prognosis is very good for unifocal skeletal system disease, and poor for multifocal disease with involvement of tissues other than bone.

HISTORICAL BACKGROUND AND TERMINOLOGY

The disease complex now known as Langerhans cell histiocytosis (LCH) has gone by a variety of designations over the past century or so. At the close of the 19th century, one of the earliest reports of one of the many manifestations of this entity was prepared by a pediatric resident, Alfred Hand,[1] who described a case of exophthalmos, polyuria, and skull destruction in a 3-year-old child in an article entitled "Polyuria and Tuberculosis." Hand suspected that this disease process was related to a specific infection, tuberculosis (although, 28 years later, he modified this thesis to make room for the possibility that other infectious agents or even a neoplasm might be implicated[2]).

A contemporary of Hand's, Erich Letterer,[3] described in the early 1920s an apparently unrelated disorder that struck children in the first few months of life (an age group younger than that studied by Hand in his reports). This disease process also contrasted with that described by Hand in that it was frequently fatal, while Hand reported on a less rapidly progressive, chronic disorder.[3]

Nearly two decades after Letterer's 1924 report, the existence of solitary skeletal lesions marked by infiltrates of histiocytes and eosinophils was reported. This skeletal disease appeared to be a benign process, and so differed in its behavior from both the Hand syndrome and the Letterer syndrome.[4]

Other reports of skeletal lesions — including some by Schüller, Christian, and Siwe — appeared between the close of the 19th century and the 1940s, but it fell to other observers to suggest the possibility that these apparently disparate entities might in reality be discrete points along a single spectrum of disease. And so, the encompassing designation of histiocytosis X (referring to the pathologic finding of large numbers of histiocyte-like cells in each of these lesions) was born.[5-7] In the 1980s it was recognized that the mononuclear cells that populate these lesions were not histiocytes of the usual type, but were in reality a particular cell type that had been recognized as a component of normal skin in the mid-19th century: the Langerhans cell.[8]

There are three well-established clinical subsets of LCH that should be defined at this time: eosinophilic granuloma (EG) of bone, Hand-Schüller-Christian (HSC) disease, and Letterer-Siwe (LS) disease.

In EG of bone, the manifestations of disease are restricted to the skeleton. Lesions are usually unifocal, but a minority may be multifocal; however, this is in general a benign process. While some morbidity can result from these skeletal defects (including pain, limitation of movement, and pathologic fracture), they present a less serious clinical picture than do the two remaining clinical types: HSC disease and LS disease. The age at diagnosis of EG may range from soon after birth to late adulthood, although most patients present between 5 and 15 years of age.

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In classic HSC disease, the typical patient is between 1 and 5 years of age and suffers from a distinct triad of manifestations: exophthalmos, diabetes insipidus, and skeletal lesions. This is a disease with chronic manifestations, as damage to the hypothalamic pituitary axis by deposits of LCH often leads to lifelong problems with diabetes insipidus.

Letterer-Siwe disease refers to cases — usually in the first year of life — of widespread involvement of multiple organ systems by deposits of LCH that may in some instances follow a rapidly progressive course that results in the child's death.

There are those who believe that these classic clinical subtypes are of more historical than practical value; indeed, their arguments have a good deal of merit.[9] In 1985, an international workshop in Philadelphia reviewed the lesions associated with histiocytosis, and the term LCH was adopted to differentiate it from neoplastic and reactive histiocytic conditions.[10] In 1987, the term LCH was also recommended to replace histiocytosis X and its eponyms (EG, HSC disease, and LS disease) by the Writing Group of the Histiocyte Society.[11] It should be conceded that there remain some observers who harbor a great dislike for the term histiocyte, and so for them this process will forever be known as Langerhans cell granulomatosis.[9] But the World Health Organization recommended adoption of the term LCH.[12]

For the purposes of this article, three clinical patterns of disease should be borne in mind: unifocal skeletal LCH (EG), multifocal LCH restricted to the skeletal system (EG), and multifocal LCH with involvement of tissues other than bone.

PATIENT CHARACTERISTICS AND CLINICAL MANIFESTATIONS

The typical patient with head and neck manifestations of LCH is a child; the skeleton is by far the organ system most frequently involved. Less often, cutaneous or pulmonary deposits of LCH are found; in only a minority of cases, other visceral sites are involved.[13] While any age group may be affected, most diagnoses of LCH are made between 5 and 20 years of age. A slight male predominance is noted, and the disease is more common in white than in black children; the incidence of LCH in the United States has been estimated at between 0.05 and 0.5 cases per 100,000 children per year.[14] The lesions of LCH may present as mass lesions, with pain, or with tenderness on physical examination.

In large series, the head and neck region is the area most often affected by LCH — either at initial presentation, or as the course of disease unfolds over time. Some estimates hold that over 60% of patients have head and neck lesions at the time of initial presentation, and that another 20% develop head and neck lesions subsequently.[15] Skull lesions are the most frequent, followed by temporal bone disease, cutaneous eruptions, mandibular and/or maxillary disease, and cervical adenopathy.[13,15-29] Isolated extraosseous LCH lesions have been reported in the skin, palatal mucosa, thymus, nose, larynx, lung, hypothalamus, thyroid, gastrointestinal tract, lymph nodes, parotid gland, kidney, and central nervous system, but all of these are extremely rare.[30-35]

The intraosseous deposits are often accompanied by an extension of the process to involve the adjacent soft tissues, and so, location of the lesion will dictate the presenting complaint: skull lesions may appear as palpable deformities, while mastoid involvement may manifest as a draining otitis or impairment of hearing, and vertebral involvement may lead to back pain or frank neurologic compromise. When deposits of LCH affect the neurohypophysis, diabetes insipidus may result. Orbital masses can produce proptosis, while mandibular and/or maxillary disease may include gingival disease and loosening of teeth. Cutaneous lesions may first be evaluated as an unexplained rash. Finally, systemic manifestations (particularly in the youngest patients affected) can include a fever, an elevated sedimentation rate, and anemia. A careful workup is mandatory for therapeutic and prognostic reasons and should include a general pediatric examination, hematologic evaluation, erythrocyte sedimentation rate, liver function tests, urinalysis, chest radiography, bone marrow biopsy, and skeletal survey.[23] There are also a few reports of "second tumors" in children with this disease.[36,37]

RADIOGRAPHIC FINDINGS

For reasons that are not clear, the skeletal lesions of LCH are more commonly seen in the flat bones than in the long bones; consequently, the skull, ribs, and pelvis account for over half of the sites of bony involvement in most series. An early lesion may have a disconcertingly aggressive appearance on plain films, presenting as a lytic focus with ill-defined margins and, in some instances, an accompanying periosteal reaction. Well-established lesions of LCH are lytic areas with sharper margins; in fact, as these lesions age, they often develop about themselves a distinct sclerotic margin (Fig 1).[14,38]

In the head and neck region in particular, calvarial lesions (particularly of the parietal bones) are more common than base of skull lesions are. Calvarial lesions may show an uneven rate of destruction of the inner table as contrasted with the outer table, yielding

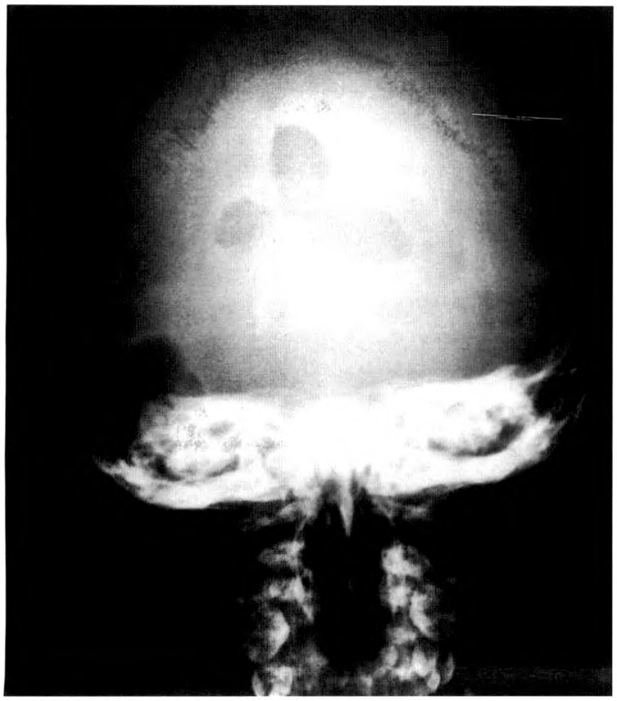

Fig 1. Head and neck manifestations of Langerhans cell histiocytosis (LCH) often include skeletal component. These osseous lesions are typically lytic and, as here, may have accompanying sclerotic rim. Temporal bone is common site for development of such lesions in head and neck region.

a beveled appearance. A central mass of residual bone — surrounded by lysis of the adjacent bone — will in some individuals produce a "bull's-eye" appearance referred to by some as a "button sequestrum." The least common radiographic manifestation of calvarial LCH is a diffuse osteopenia.

When the base of the skull is affected, the temporal bone is a particularly common site of occurrence. Less frequently, deposits of LCH are found in the vicinity of the sella or the orbits.

The mandible[39] and maxilla are sometimes involved in LCH. This involvement may lead to presentation with dental complaints (which may manifest radiographically as "floating teeth," the result of lysis of the adjacent alveolar bone as a result of deposits of LCH).[40] Vertebral body lesions may develop in the cervical spine region, although both the thoracic spine and the lumbar spine are more often involved than is the cervical spine; vertebral lesions typically produce the characteristic flattening of the affected body known as a "vertebra plana."

In each of the situations noted above, the osseous lesion may be accompanied by a soft tissue mass — a phenomenon often better appreciated on computed tomography or magnetic resonance scanning. Lesions inapparent on plain films may be identified as areas of increased uptake on radionuclide scanning.

PATHOLOGIC FINDINGS

Gross lesions of LCH vary in color from yellow to tan to red (hemorrhagic); areas of softening suggestive of necrotic foci may be present.

The light microscopic features of LCH were well described over half a century ago; whether unifocal or multifocal, and whether skeletal or extraskeletal, lesions of LCH all have in common that they include, on microscopic examination, a proliferation of mononuclear cells, the Langerhans cells.[41] These mono-

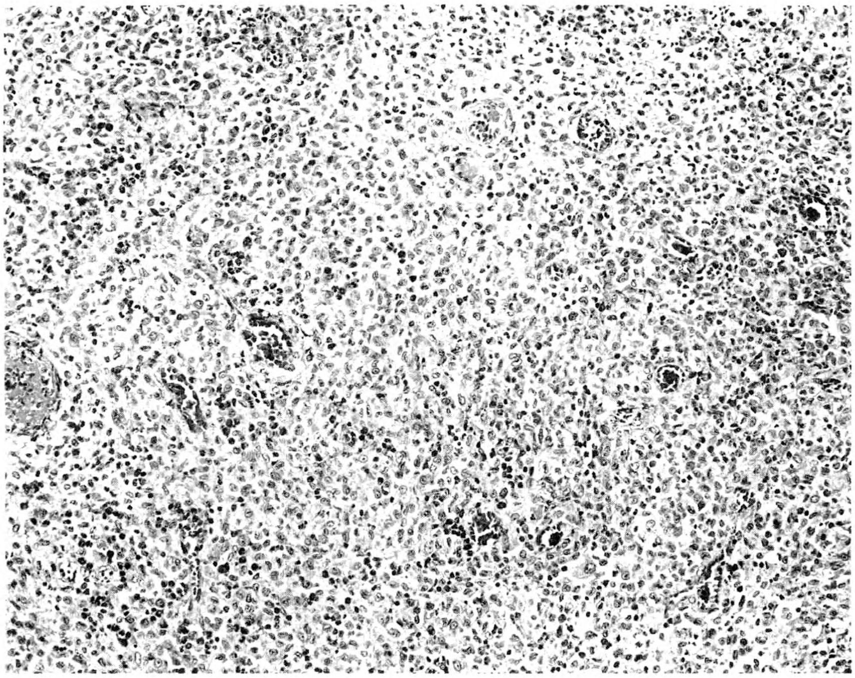

Fig 2. Low-power appearance of LCH is marked by polymorphous cellular infiltrate with single and multinucleated histiocyte-like cells intimately admixed with eosinophils (H & E, original ×110).

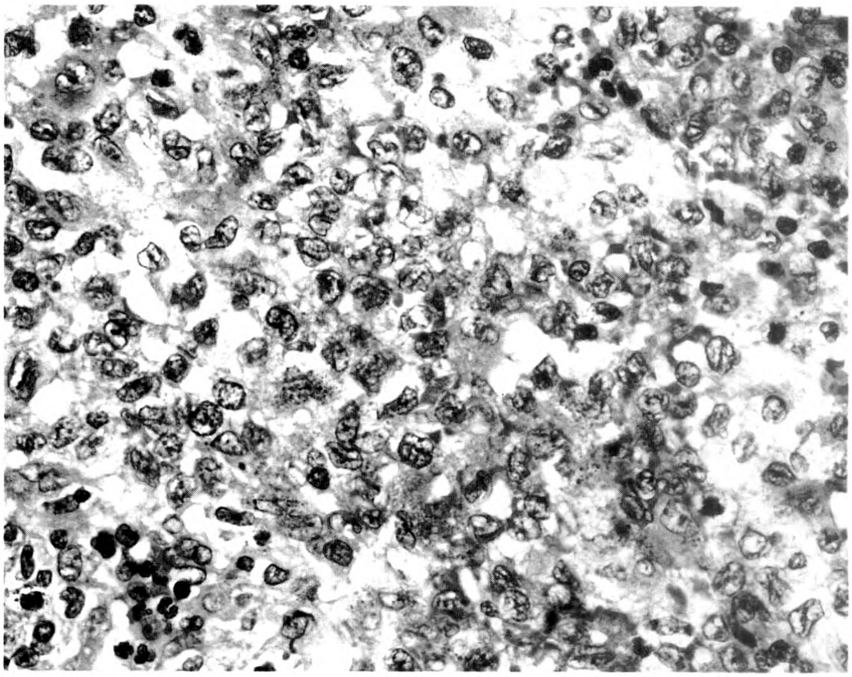

Fig 3. Diagnostic Langerhans cells are large mononuclear cells that bear close resemblance to ordinary histiocytes (H & E, original ×420). Cytologically, Langerhans cells differ slightly from histiocytes of usual type, in that Langerhans have more convoluted, folded, or clefted nuclei.

nuclear cells are, in turn, accompanied by a variable proportion of multinucleated giant cells and eosinophils (and hence the name EG). While the giant cells and/or eosinophils are not required for the diagnosis and may even be absent, the Langerhans cells are invariably present and so represent the diagnostic component of a lesion of LCH. It has been noted in the past that it is not possible, on light microscopic grounds, to distinguish among the lesions of EG, HSC disease, and LS disease; they are, instead, of a single microscopic appearance.

A low-power view of a typical deposit of LCH is a polymorphous, variegated arrangement of mononuclear histiocyte-like cells (the Langerhans cells), scattered multinucleated giant cells, and bilobed eosinophils (Fig 2). There may, in addition, be variable numbers of small mature lymphocytes and plasma cells scattered throughout the background. On closer scrutiny, the Langerhans cells prove to contain nuclei that are convoluted and folded — a degree of nuclear complexity that, incidentally, is not typical of histiocytes of the usual type (Fig 3).

Perhaps the most significant advance in our understanding of the pathologic aspects of LCH came with

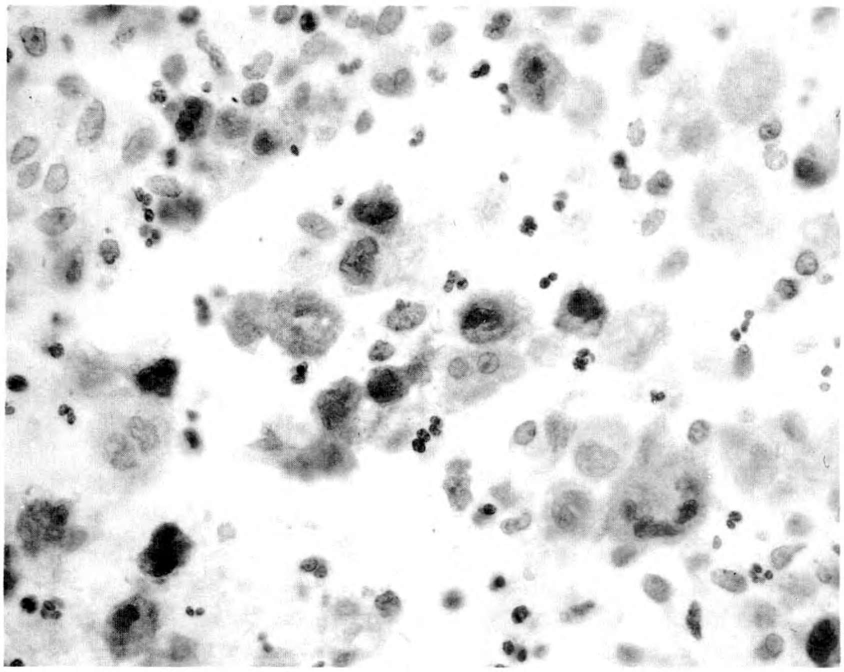

Fig 4. Whenever confirmation of light microscopic diagnosis of LCH is required, most pathologists rely on immunohistochemical staining for S-100 protein (antibody to S-100 protein, original ×420). As seen here, S-100 protein positivity among Langerhans cells is nuclear reaction. Histiocytes of usual type are S-100 protein–negative.

TABLE 1. CLINICAL AND RADIOGRAPHIC DIFFERENTIAL DIAGNOSIS OF LANGERHANS CELL HISTIOCYTOSIS

Langerhans cell histiocytosis
Aural polyp
Chronic otitis media or external otitis
Mastoiditis
Cholesterol granuloma
Cholesteatoma
Sinusitis
Dermatitis (eczema, atopy)
Osteomyelitis
Severe combined immunodeficiency
Graft-versus-host disease (in appropriate context)
Infection-associated or familial hemophagocytic syndrome
Malignant lymphoma
Ewing's sarcoma
Metastasis (particularly neuroblastoma)

the recognition that the mononuclear cells that typify lesions of LCH are not histiocytes (macrophages) of the ordinary type, but rather are members of the dendritic system and so are involved in the cell-mediated immune response. The particular nature of the diagnostic cells — the Langerhans cells — lends itself to pathologic confirmation of a diagnosis of LCH. It happens that Langerhans cells are positive on immunohistochemical study for adenosine triphosphatase, α-D-mannosidase, and S-100 protein, while histiocytes of the usual type (with which the Langerhans cells are easily confused) are S-100 protein–negative (Fig 4). The Langerhans cells may be specifically identified by electron microscopy, which reveals characteristic rod-shaped Birbeck granules in their cytoplasm.[42] The Writing Group of the Histiocyte Society[11] suggested that a "presumptive" diagnosis should be confirmed by electron microscopic demonstration of Birbeck granules to become a "definitive" diagnosis. Immunohistochemical investigations may assist the morphologic diagnosis based on conventional light microscopy.

COURSE OF DISEASE AND TREATMENT

It was not too many years ago that multifocal LCH was treated aggressively with highly toxic chemotherapeutic agents (including methotrexate, vincristine, and cyclophosphamide) and radiotherapy, as would be appropriate for any disseminated malignant tumor. Recently, however, increasing pressure has been brought on clinicians to employ less aggressive treatment regimens for the control of what some observers believe to be a nonneoplastic disease.[10,29,43,44] It is clear that children below the age of 2 years who develop multifocal disease affecting the viscera as well as the skeleton are at the greatest risk of suffering a fulminant course that can result in death; older children seem better able to cope with this disease, and in them it follows a more benign course.

In older children with limited disease, it has long been known that some lesions will regress spontaneously without therapy, while other lesions appear to respond to steroids, low-dose radiotherapy, or simple incisional biopsy or curettage, and so this (less aggressive) therapy is currently regarded as the approach of choice when possible. At the same time, it must be acknowledged that the fulminant course of disease, seen particularly in some very young patients, is not amenable to such an approach; in such patients, chemotherapeutic agents have been employed with success (an observation, incidentally, that is cited in support of their arguments by those who regard LCH as a malignancy).

One common theme seems to link together many of the reported cases of death from LCH — namely, the attribution of the immediate cause of death to a secondary complicating infection. Such an observation raises the possibility that the historically identified clinical groups — youngest with the worst prognosis, oldest with the best prognosis — may actually reflect the relative maturity of the affected child's immune system. Thus, the youngest patients, with the least-developed immune mechanisms, are those most likely to fall prey to the LCH and its attendant predisposition to secondary infections, while older patients are better equipped to ward off these insults and so are much more likely to survive their diseases. Should this prove to be the case, then it is likely that the thrust of therapy for LCH will continue to evolve in a "minimalist" direction, with the aggressive toxic agents held in reserve for only those children whose rapidly progressive course of disease calls for some rapid response (coupled, of course, with a vigilant watch for and treatment of complicating secondary infections).

DIFFERENTIAL DIAGNOSIS

From a physical examination and radiographic vantage point, the skeletal lesions of LCH may suggest a wide range of possibilities, including aural polyps, granulation tissue,[45] chronic otitis media,[46] external otitis, mastoiditis, cholesteatoma (in patients with temporal bone disease), sinusitis, a dermatitis such as eczema (in patients with cutaneous manifestations), osteomyelitis, hemangioma, fibrous dysplasia, meningioma, leukemia, Ewing's sarcoma, and metastasis (particularly metastatic neuroblastoma) (Table 1).

In patients with disseminated multisystem disease, consideration might be given in the clinical differential diagnosis to osteomyelitis, infection-associated

TABLE 2. PATHOLOGIC DIFFERENTIAL DIAGNOSIS OF LANGERHANS CELL HISTIOCYTOSIS

Langerhans cell histiocytosis
Osteomyelitis
Juvenile xanthogranuloma
Mycobacterial infection
Reticulohistiocytoma
Urticaria pigmentosa
Dermatopathic lymphadenopathy
Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)
Malignant lymphoma
Hodgkin's disease

or familial hemophagocytic syndrome, malignant lymphoma, and graft-versus-host disease.

From a pathologic perspective, the differential diagnosis may touch upon such disparate entities as osteomyelitis, juvenile xanthogranuloma, mycobacterial infection, reticulohistiocytoma, urticaria pigmentosa, dermatopathic lymphadenopathy, sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease), malignant lymphoma, and Hodgkin's disease (Table 2).[47]

CONCLUSIONS

As our understanding of LCH has evolved over the past century, its essential nature has been variously attributed to the influence of an infectious agent, trauma, a benign neoplasm, a malignant neoplasm, and, finally, an unknown process, perhaps similar to sarcoidosis. There is no doubt that some reports — particularly those dating from the early part of this century — have included both cases of LCH and cases of other disease processes together as examples of LCH, which of necessity has clouded our understanding of this entity. Nevertheless, current thinking about LCH suggests that its pathogenesis is, in some fashion, related to a disorder of the immune response.[44] It is currently a matter of some debate whether this abnormal immune response, with a proliferation of Langerhans cells to yield discrete mass lesions, is an unwanted response to some antigenic stimulus (perhaps infectious?) or a frankly neoplastic proliferation of Langerhans cells. The proliferation, differentiation, and activation of normal Langerhans cells are controlled by granulocyte-macrophage colony-stimulating factor (GM-CSF) in vitro.[48] One study suggests that GM-CSF may be implicated in the pathogenesis of LCH.[48]

The prognosis is very good for unifocal, skeletal system disease, and poor for multifocal disease with involvement of tissues other than bone.

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Lorabid® (loracarbef, USP)

Pulvules®, 200 mg and 400 mg

For Oral Suspension, 100 mg or 200 mg/5mL (50-mL and 100-mL size)

Brief Summary. Consult the package insert for complete prescribing information.

INDICATIONS AND USAGE: Lorabid is a synthetic β-lactam antibiotic of the carbacephem class for oral administration. Lorabid is indicated in the following mild to moderate infections caused by susceptible strains of designated microorganisms.

Secondary Bacterial Infection of Acute Bronchitis caused by *Streptococcus pneumoniae, Haemophilus influenzae* (including β-lactamase-producing strains), or *Moraxella (Branhamella) catarrhalis* (including β-lactamase-producing strains).

Acute Bacterial Exacerbations of Chronic Bronchitis caused by *S. pneumoniae, H. influenzae* (including β-lactamase-producing strains), or *M. catarrhalis* (including β-lactamase-producing strains).

Pneumonia caused by *S. pneumoniae, or H. influenzae* (non-β-lactamase-producing strains only).

Otitis Media* caused by *S. pneumoniae, H. influenzae* (including β-lactamase-producing strains), *M. catarrhalis* (including β-lactamase-producing strains), or *Streptococcus pyogenes.*

Acute Maxillary Sinusitis* caused by *S. pneumoniae, H. influenzae* (non-β-lactamase-producing strains only), or *M. catarrhalis* (including β-lactamase-producing strains).

*NOTE: In a patient population with significant numbers of β-lactamase-producing organisms, loracarbef's clinical cure and bacteriological eradication rates were somewhat less than those observed with a product containing a β-lactamase inhibitor. Lorabid's decreased potential for toxicity compared to products containing β-lactamase inhibitors along with the susceptibility patterns of the common microbes in a given geographic area should be taken into account when considering the use of an antimicrobial (see **CLINICAL STUDIES** section). For information on use in pediatric patients, see **PRECAUTIONS**.

Pharyngitis and Tonsillitis caused by *S. pyogenes.* (The usual drug of choice in the treatment and prevention of streptococcal infections, including the prophylaxis of rheumatic fever, is penicillin administered by the intramuscular route. Lorabid is generally effective in the eradication of *S. pyogenes* from the nasopharynx; however, data establishing the efficacy of Lorabid in the subsequent prevention of rheumatic fever are not available at present.)

Uncomplicated Skin and Skin Structure Infections caused by *Staphylococcus aureus* (including penicillinase-producing strains) or *S. pyogenes.* Abscesses should be surgically drained as clinically indicated.

Uncomplicated Urinary Tract Infections (cystitis) caused by *Escherichia coli* or *Staphylococcus saprophyticus**.

NOTE: In considering the use of Lorabid in the treatment of cystitis, Lorabid's lower bacterial eradication rates and lower potential for toxicity should be weighed against the increased eradication rates and increased potential for toxicity demonstrated by some other classes of approved agents (*see* **CLINICAL STUDIES** section).

Uncomplicated Pyelonephritis caused by *E. coli.*

*Although treatment of infections due to this organism in the organ system demonstrated a clinically acceptable overall outcome, efficacy was studied in fewer than 10 infections.

CONTRAINDICATION: Known allergy to loracarbef or cephalosporin-class antibiotics.

WARNINGS: BECAUSE CROSS-HYPERSENSITIVITY CAN OCCUR AMONG β-LACTAMS, LORABID SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS AND DISCONTINUED IF AN ALLERGIC REACTION OCCURS.

Pseudomembranous colitis has been reported with nearly all antibacterial agents and should be considered in differential diagnosis of antibiotic-associated diarrhea.

PRECAUTIONS: Lorabid may be administered to patients with impaired renal function. Total daily dosage should be reduced in patients with known or suspected renal impairment because of the possibility of high and/or prolonged plasma concentrations.

Loracarbef should be given cautiously to patients receiving diuretics concurrently.

Prolonged use may result in overgrowth of nonsusceptible organisms.

Loracarbef should be given cautiously to patients with a history of colitis.

Renal excretion of β-lactams is inhibited by probenecid and resulted in about an 80% increase in the AUC for loracarbef.

Safety and effectiveness have not been determined in pregnancy, lactation, and pediatric patients under 6 months of age. Caution should be exercised in prescribing Lorabid for these patients.

In geriatric patients who received the usual recommended adult doses in clinical studies, efficacy and safety were comparable to results in nongeriatric adult patients.

The safety and efficacy of Lorabid have been established for children aged 6 months to 12 years for acute maxillary sinusitis based upon its approval in adults, using as support the following data: pharmacokinetic and safety data in adults and children; clinical and microbiologic data in acute maxillary sinusitis (adults) and acute otitis media with effusion (children); postmarketing adverse events surveillance.

ADVERSE REACTIONS: Most adverse reactions in clinical trials were mild and transient. Only 1.5% of patients discontinued because of drug-related reactions, the most common of which were diarrhea, abdominal pain, and skin rashes.

All Patients

The incidence of the following adverse events was less than 1%, except as otherwise noted:

Gastrointestinal: Diarrhea, 4.1%; nausea, 1.9%; vomiting, 1.4%; abdominal pain, 1.4%; and anorexia.

Hypersensitivity: Skin rashes (1.2%), urticaria, pruritus, and erythema multiforme.

Central Nervous System: Headache (2.9%), somnolence, nervousness, insomnia, and dizziness.

Hemic and Lymphatic Systems: Transient thrombocytopenia, leukopenia, and eosinophilia.

Hepatic: Transient elevations in AST (SGOT), ALT (SGPT), and alkaline phosphatase.

Renal: Transient elevations in BUN and creatinine.

Cardiovascular System: Vasodilatation.

Genitourinary: Vaginitis: (1.3%), vaginal moniliasis (1.1%).

As with other β-lactam antibiotics, the following potentially severe adverse experiences have been reported rarely with loracarbef in worldwide postmarketing surveillance: anaphylaxis, hepatic dysfunction including cholestasis, prolongation of the prothrombin time with clinical bleeding in patients taking anticoagulants, and Stevens-Johnson syndrome.

Pediatric Patients

The incidences of several adverse events were significantly different in the pediatric population versus the adult population respectively as follows: Diarrhea (5.8% vs 3.6%); nausea (0.0% vs 2.5%); vomiting (3.3% vs 0.5%); anorexia (2.3% vs. 0.3%); headache (0.9% vs. 3.2%); somnolence (2.1% vs 0.4%); rhinitis (6.3% vs 1.6%); rash (2.9% vs 0.7%).

β-Lactam Antimicrobial Class Labeling:

The following adverse reactions and altered laboratory test results have been reported in patients treated with β-lactam antibiotics:

Adverse Reactions—Allergic reactions, aplastic anemia, hemolytic anemia, hemorrhage, agranulocytosis, toxic epidermal necrolysis, renal dysfunction, and toxic nephropathy. As with other β-lactam antibiotics, serum sickness-like reactions have been reported rarely with loracarbef.

Altered Laboratory Tests—Increased prothrombin time, positive direct Coombs' test, elevated LDH, pancytopenia, and neutropenia.

ERDOSAGE: Hemodialysis has been shown to be effective in hastening the elimination of arbef from plasma in patients with chronic renal failure.

E AND ADMINISTRATION: Lorabid is administered orally either at least 1 hour prior to at least 2 hours after eating.

Population/Infection	Dosage (mg)	Duration (days)
ADULTS (≥13 years)		
Secondary Bacterial Infection of Acute Bronchitis	200-400 q12h	7
Acute Bacterial Exacerbation of Chronic Bronchitis	400 q12h	7
Pneumonia	400 q12h	14
Pharyngitis/Tonsillitis	200 q12h	10
Sinusitis	400 q12h	10
(*See* **CLINICAL STUDIES** *and* **INDICATIONS AND USAGE** for further information.)		
Uncomplicated Skin and Skin Structure Infections	200 q12h	7
Uncomplicated cystitis	200 q24h	7
(*See* **CLINICAL STUDIES** *and* **INDICATIONS AND USAGE** for further information.)		
Uncomplicated pyelonephritis	400 q12h	14
PEDIATRIC PATIENTS (6 months to 12 yrs)		
Acute Otitis Media*	30 mg/kg/day q12h (divided doses)	10
(*See* **CLINICAL STUDIES** *and* **INDICATIONS AND USAGE** for further information.)		
Acute Maxillary Sinusitis	30 mg/kg/day q12h (divided doses)	10
(*See* **CLINICAL STUDIES** *and* **INDICATIONS AND USAGE** for further information.)		
Pharyngitis/Tonsillitis	15 mg/kg/day q12h (divided doses)	10
Impetigo	15 mg/kg/day q12h (divided doses)	7

*Clinical studies of otitis media were conducted with the suspension formulation only. Therefore, the capsule should not be substituted for the suspension in the treatment of otitis media.

CLINICAL STUDIES:

Loracarbef (L) vs β-Lactamase Inhibitor (C) in Acute Otitis Media (US)

Efficacy: A study of acute otitis media performed in a population with a significant incidence of β-lactamase-producing organisms compared loracarbef with a β-lactamase inhibitor. Using very strict evaluability and microbiologic/clinical response criteria at the 10- to 16-day posttherapy follow-up, the following presumptive bacterial eradication/clinical cure outcomes (success rates) were obtained:

Pathogen	% Due to Pathogens (N = 204)	Success Rate
S. pneumoniae	42.6%	L equivalent to C
H. influenzae	30.4%	L 9% less than C
M. catarrhalis	20.6%	L 19% less than C
S. pyogenes	6.4%	L equivalent to C
Overall	100.0%	L 12% less than C

Safety: The incidences of the most common adverse events were clinically and statistically significantly higher in the control group versus the loracarbef group.

Event	Loracarbef	Control
Diarrhea	15%	26%
Rash*	8%	15%

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*Primarily in the diaper area in young pediatric patients.

Loracarbef (L) vs Amoxicillin (A) in Acute Otitis Media (Europe)

Efficacy: A study of acute otitis media performed in a population with a lower incidence of β-lactamase-producing organisms than that usually seen in US trials compared loracarbef to amoxicillin. Using very strict evaluability and microbiologic/clinical response criteria at the 10- to 16-day posttherapy follow-up, the following presumptive bacterial eradication/clinical cure outcomes (success rates) were obtained:

Pathogen	% Due to Pathogen (N = 291)	Success Rate
S. pneumoniae	51.5%	L equivalent to A
H. influenzae	29.2%	L 14% greater than A
M. catarrhalis	15.8%	L 31% greater than A
S. pyogenes	3.4%	L equivalent to A
Overall	100.0%	L equivalent to A

Loracarbef (L) vs Doxycycline (D) in Acute Maxillary Sinusitis (Europe)

Efficacy: A study of acute maxillary sinusitis performed in a population with a lower incidence of β-lactamase-producing organisms than that usually seen in US trials compared loracarbef with doxycycline. Using very strict evaluability (sinus-puncture) criteria and microbiologic/clinical response criteria at the 1- to 2-week posttherapy follow-up, the following presumptive bacterial eradication/clinical cure outcomes (success rates) were obtained:

Pathogen	% Due to Pathogen (N = 210)	Success Rate
S. pneumoniae	47.6%	L equivalent to D
H. influenzae	41.4%	L equivalent to D
M. catarrhalis	11.0%	L equivalent to D
Overall	100.0%	L equivalent to D

Loracarbef (L) vs Cefaclor (C) in Uncomplicated Cystitis Study (US)

Efficacy: A study of cystitis compared loracarbef with cefaclor. Using very strict evaluability criteria and microbiologic/clinical response criteria at the 5- to 9-day posttherapy follow-up, the following bacterial eradication rates were obtained:

Pathogen	% Due to Pathogen (N = 186)	Eradication Rate
E. coli	77.4%	L 4% greater than C (L = 80%)
Other major *Enterobacteriaceae*	12.5%	L equivalent to C (L = 61%)
S. saprophyticus	3.8%	L equivalent to C

Loracarbef (L) vs Quinolone (Q) in Uncomplicated Cystitis (Europe)

Efficacy: A study of cystitis compared loracarbef with an oral quinolone. Using very strict evaluability criteria and microbiologic/clinical response criteria at the 5- to 9-day posttherapy follow-up, the following bacterial eradication rates were obtained:

Pathogen	% Due to Pathogens (N = 189)	Eradication Rate
E. coli	82.0%	L 7% less than Q (L = 81%)
Other major *Enterobacteriaceae*	10.1%	L 32% less than Q (L =50%)

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